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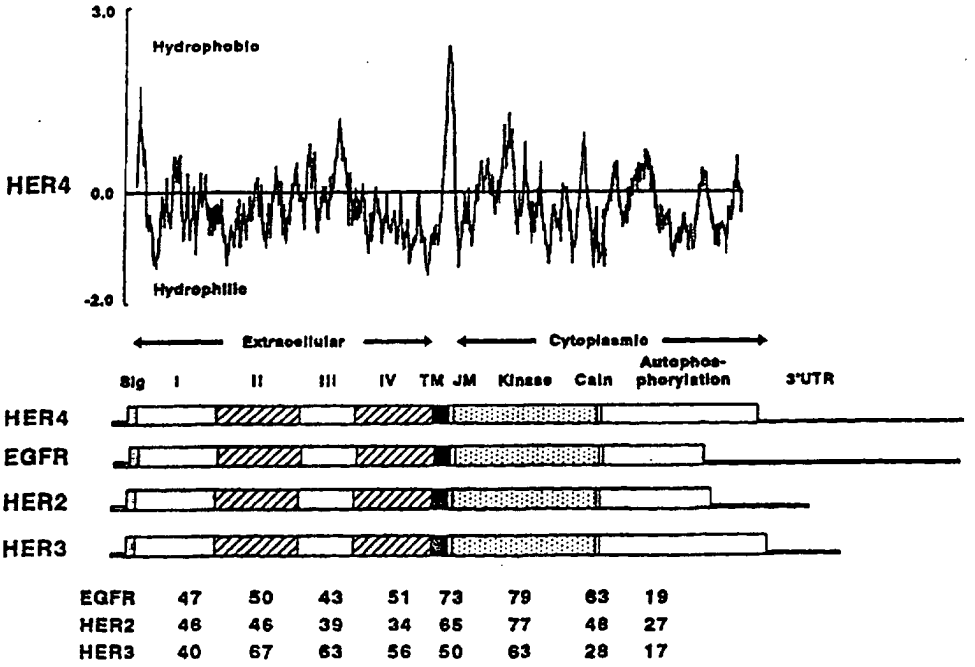

EGFR	47	50	43	51	73	79	63	19
HER2	46	46	39	34	65	77	48	27
HER3	40	67	63	56	50	63	28	17

(57) Abstract

The molecular cloning, expression, and biological characteristics of a novel receptor tyrosine kinase related to the epidermal growth factor receptor, termed HER4/p180^{erbB4}, are described. An HER4 ligand capable of inducing cellular differentiation of breast cancer cells is also disclosed. In view of the expression of HER4 in several human cancers and in certain tissues of neuronal and muscular origin, various diagnostic and therapeutic uses of HER4-derived and HER4-related biological compositions are provided.

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HER4 HUMAN RECEPTOR TYROSINE KINASE

This application is a continuation-in-part of United States Application Serial No. 08/150,704, filed November 10, 1993, which is a continuation-in-part of United States Application Serial No. 07/981,165, filed November 24, 1992, each of which applications are incorporated herein in their entireties.

1. Introduction

The present invention is generally directed to a novel receptor tyrosine kinase related to the epidermal growth factor receptor, termed HER4/p180^{erbB4} ("HER4"), and to novel diagnostic and therapeutic compositions comprising HER4-derived or HER4-related biological components. The invention is based in part upon applicants discovery of human HER4, its complete nucleotide coding sequence, and functional properties of the HER4 receptor protein. More specifically, the invention is directed to HER4 biologics comprising, for example, polynucleotide molecules encoding HER4, HER4 polypeptides, anti-HER4 antibodies which recognize epitopes of HER4 polypeptides, ligands which interact with HER4, and diagnostic and therapeutic compositions and methods based fundamentally upon such molecules. In view of the expression of HER4 in several human cancers and in certain tissues of neuronal and muscular origin, the present invention provides a framework upon which effective biological therapies may be designed. The invention is hereinafter described in detail, in part by way of experimental examples specifically illustrating various aspects of the invention and particular embodiments thereof.

2. Background of the Invention

Cells of virtually all tissue types express transmembrane receptor molecules with intrinsic tyrosine kinase activity through which various growth and differentiation factors mediate a range of biological effects (reviewed in Aaronson, 1991, Science 254:1146-52). Included in this group of receptor tyrosine kinases (RTKs) are the receptors for polypeptide growth factors such as epidermal growth factor (EGF), insulin, platelet-derived growth factor (PDGF), neurotrophins (*i.e.*, NGF), and fibroblast growth factor (FGF). Recently, the ligands for several previously-characterized receptors have been identified, including ligands for c-kit (steel factor), met (hepatocyte growth factor), trk (nerve growth factor) (see, respectively, Zsebo *et al.*, 1990, Cell 63:195-201; Bottardo *et al.*, 1991, Science 251:802-04; Kaplan *et al.*, 1991, Nature 350:158-160). In addition, the soluble factor NDF, or heregulin-alpha (HRG-α), has been identified as the ligand for HER2, a receptor which is highly related to HER4 (Wen *et al.*, 1992, Cell 69:559-72; Holmes *et al.*, 1992, Science 256:1205-10).

The heregulins are a family of molecules that were first isolated as specific ligands for HER2 (Wen, *et al.*, 1992, Cell, 69:559-572; Holmes *et al.*, 1992, Science 256:1205-1210; Falls *et al.*, 1993, Cell 72:801-815; and Marchionni *et al.*, 1993, Nature 362:312-318). A rat homologue was termed Neu differentiation factor (NDF) based on its ability to induce differentiation of breast cancer cells through its interaction with HER2/Neu (Wen *et al.*, *supra*). Heregulin also appears to play an important role in development and maintenance of the nervous system based on its abundant expression in cells of neuronal

origin and on the recognition that alternatively spliced forms of the heregulin gene encode for two recently characterized neurotrophic activities. One neural-derived factor is termed acetylcholine receptor inducing activity (ARIA)(Falls *et al.*, *supra*). This heregulin isoform is responsible for stimulation of neurotransmitter receptor synthesis during formation of the neuromuscular junction. A second factor is called glial growth factor (GGF) reflecting the proliferative affect this molecule has on glial cells in the central and peripheral nervous system (Marchionni *et al.*, *supra*). Additional, less well characterized molecules that appear to be isoforms of heregulin, include p45, gp30, and p75 (Lupu *et al.*, 1990, Science 249:1552-1555; and Lupu *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:2287-2291).

Several HER2-neutralizing antibodies fail to block heregulin activation of human breast cancer cells. Heregulin only activates tyrosine phosphorylation of HER2 in cells of breast, colon, and neuronal origin, and not in fibroblasts or ovarian cell lines that overexpress recombinant HER2 (Peles *et al.*, 1993, EMBO J. 12:961-971).

Biological relationships between various human malignancies and genetic aberrations in growth factor-receptor tyrosine kinase signal pathways are known to exist. Among the most notable such relationships involve the EGF receptor (EGFR) family of receptor tyrosine kinases (see Aaronson, *supra*). Three human EGFR-family members have been identified and are known to those skilled in the art: EGFR, HER2/p185^{erbB2} and HER3/p160^{erbB3} (see, respectively, Ullrich *et al.*, 1984, Nature 309:418-25; Coussens *et al.*, 1985, Science 230:1132-39; Plowman *et al.*, 1990, Proc. Natl. Acad.

Sci. U.S.A. 87:4905-09). EGFR-related molecules from other species have also been identified.

The complete nucleotide coding sequence of other EGFR-family members has also been determined from other organisms including: the drosophila EGFR ("DER": Livneh *et al.*, 1985, Cell 40:599-607), nematode EGFR ("let-23": Aroian *et al.*, 1990, Nature 348:693-698), chicken EGFR ("CER": Lax *et al.*, 1988, Mol. Cell. Biol. 8:1970-1978), rat EGFR (Petch *et al.*, 1990, Mol. Cell. Biol. 10:2973-2982), rat HER2/Neu (Bargmann *et al.*, 1986, Nature, 319:226-230) and a novel member isolated from the fish and termed *Xiphophorus* melanoma related kinase ("Xmrk": Wittbrodt *et al.*, 1989, Nature 342:415-421). In addition, PCR technology has led to the isolation of other short DNA fragments that may encode novel receptors or may represent species-specific homologs of known receptors. One recent example is the isolation tyro-2 (Lai, C. and Lemke, G., 1991, Neuron 6:691-704) a fragment encoding 54 amino acids that is most related to the EGFR family.

Overexpression of EGFR-family receptors is frequently observed in a variety of aggressive human epithelial carcinomas. In particular, increased expression of EGFR is associated with more aggressive carcinomas of the breast, bladder, lung and stomach (see, for example, Neal *et al.*, 1985, Lancet 1:366-68; Sainsbury *et al.*, 1987, Lancet 1:1398-1402; Yasui *et al.*, 1988, Int. J. Cancer 41:211-17; Veale *et al.*, 1987, Cancer 55:513-16). In addition, amplification and overexpression of HER2 has been associated with a wide variety of human malignancies, particularly breast and ovarian carcinomas, for which a strong correlation between HER2 overexpression and poor clinical prognosis and/or increased relapse probability have been established (see, for example,

Slamon *et al.*, 1987, Science 235:177-82, and 1989, Science 244:707-12). Overexpression of HER2 has also been correlated with other human carcinomas, including carcinoma of the stomach, endometrium, salivary gland, bladder, and lung (Yokota *et al.*, 1986, Lancet 1:765-67; Fukushigi *et al.*, 1986, Mol. Cell. Biol. 6:955-58; Yonemura *et al.*, 1991, Cancer Res. 51:1034; Weiner *et al.*, 1990, Cancer Res. 50:421-25; Geurin *et al.*, 1988, Oncogene Res. 3:21-31; Semba *et al.*, 1985, Proc. Natl. Acad. Sci. U.S.A. 82:6497-6501; Zhau *et al.*, 1990, Mol. Carcinog. 3:354-57; McCann *et al.*, 1990, Cancer 65:88-92). Most recently, a potential link between HER2 overexpression and gastric carcinoma has been reported (Jaehne *et al.*, 1992, J. Cancer Res. Clin. Oncol. 118:474-79). Finally, amplified expression of the recently described HER3 receptor has been observed in a wide variety of human adenocarcinomas (Poller *et al.*, 1992, J. Path 168:275-280; Krause *et al.*, 1989, Proc. Natl. Acad. Sci. U.S.A. 86:9193-97; European Patent Application No. 91301737, published 9.4.91, EP 444 961).

Several structurally related soluble polypeptides capable of specifically binding to EGFR have been identified and characterized, including EGF, transforming growth factor-alpha (TGF-α), amphiregulin (AR), heparin-binding EGF (HB-EGF), and vaccinia virus growth factor (VGF) (see, respectively, Savage *et al.*, 1972, J. Biol. Chem. 247:7612-21; Marquardt *et al.*, 1984, Science 223:1079-82; Shoyab *et al.*, 1989, Science 243:1074-76; Higashiyama *et al.*, 1991, Science 251:936-39; Twardzik *et al.*, 1985, Proc. Natl. Acad. Sci. U.S.A. 82:5300-04). Despite the close structural relationships among receptors of the EGFR-family, none of these ligands has been conclusively shown to interact with HER2 or HER3.

Recently, several groups have reported the identification of specific ligands for HER2. Some of these ligands, such as gp30 (Lupu *et al.*, 1990, Science 249:1552-55; Bacus *et al.*, 1992, Cell Growth and Differentiation 3:401-11) interact with both EGFR and HER2, while others are reported to bind specifically to HER2 (Wen *et al.*, 1992, Cell 69:559-72; Peles *et al.*, 1992, Cell 69:205-16; Holmes *et al.*, 1992, Science 256:1205-10; Lupu *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:2287-91; Huang *et al.*, 1992, J. Biol. Chem. 276:11508-121). The best characterized of these ligands are neu differentiation factor (NDF) purified and cloned from ras-transformed Rat1-EJ cells (Wen *et al.*, Peles *et al.*, *supra*), and the heregulins (HRG-α, -β1, -β2, -β3), purified and cloned from human MDA-MB-231 cells (Holmes *et al.*, *supra*). NDF and HRG-α share 93% sequence identity and appear to be the rat and human homologs of the same protein. Both of these proteins are similar size (44-45 kDa), increase tyrosine phosphorylation of HER2 in MDA-MB-453 cells and not the EGF-receptor, and have been reported to bind to HER2 in cross-linking studies on human breast cancer cells. In addition, NDF has been shown to induce differentiation of human mammary tumor cells to milk-producing, growth-arrested cells, whereas the heregulin family have been reported to stimulate proliferation of cultured human breast cancers cell monolayers.

Interestingly, although members of the heregulin family are capable of stimulating tyrosine phosphorylation of HER2 in many mammary carcinoma cell lines, they are not able to act on this receptor in the ovarian carcinoma cell line SKOV3 or in HER2 transfected fibroblasts (Peles *et al.*, 1993, EMBO J. 12:961-971). These observations indicated the

existence of other receptors for heregulin responsible for the activation of HER2. Such cross-activation between members of the receptor tyrosine kinase family has been already reported and is believed to arise from a ligand induced receptor heterodimerization event (Wada *et al.*, 1990, Cell 61:1339-1347). Recently, it has been reported that HER3 binds heregulin (Carraway *et al.*, 1994, J. Biol. Chem. 269:14303-14306), and in fact, this receptor seems to be involved in the heregulin-mediated tyrosine kinase activation of HER2 (Carraway *et al., supra*; Sliwkowski *et al.*, 1994, J. Biol. Chem. 269:14661-14665).

The means by which receptor polypeptides transduce regulatory signals in response to ligand binding is not fully understood, and continues to be the subject of intensive investigation. However, important components of the process have been uncovered, including the understanding that phosphorylation of and by cell surface receptors hold fundamental roles in signal transduction. In addition to the involvement of phosphorylation in the signal process, the intracellular phenomena of receptor dimerization and receptor crosstalk function as primary components of the circuit through which ligand binding triggers a resulting cellular response. Ligand binding to transmembrane receptor tyrosine kinases induces receptor dimerization, leading to activation of kinase function through the interaction of adjacent cytoplasmic domains. Receptor crosstalk refers to intracellular communication between two or more proximate receptor molecules mediated by, for example, activation of one receptor through a mechanism involving the kinase activity of the other. One particularly relevant example of such a phenomenon

is the binding of EGF to the EGFR, resulting in activation of the EGFR kinase domain and cross-phosphorylation of HER2 (Kokai *et al.*, 1989, Cell 58:287-92; Stern *et al.*, 1988, EMBO J. 7:995-1001; King *et al.*, 1989, Oncogene 4:13-18).

3. Summary of the Invention

HER4 is the fourth member of the EGFR-family of receptor tyrosine kinases and is likely to be involved not only in regulating normal cellular function but also in the loss of normal growth control associated with certain human cancers. In this connection, HER4 appears to be closely connected with certain carcinomas of epithelial origin, such as adenocarcinoma of the breast. As such, its discovery, and the elucidation of the HER4 coding sequence, open a number of novel approaches to the diagnosis and treatment of human cancers in which the aberrant expression and/or function of this cell surface receptor is involved.

The complete nucleotide sequence encoding the prototype HER4 polypeptide of the invention is disclosed herein, and provides the basis for several general aspects of the invention hereinafter described. Thus, the invention includes embodiments directly involving the production and use of HER4 polynucleotide molecules. In addition, the invention provides HER4 polypeptides, such as the prototype HER4 polypeptide disclosed and characterized in the sections which follow. Polypeptides sharing nearly equivalent structural characteristics with the prototype HER4 molecule are also included within the scope of this invention. Furthermore, the invention includes polypeptides which interact with HER4 expressed on the surface of certain cells thereby

affecting their growth and/or differentiation. The invention is also directed to anti-HER4 antibodies, which have a variety of uses including but not limited to their use as components of novel biological approaches to human cancer diagnosis and therapy provided by the invention.

The invention also relates to the identification of HER4 ligands and methods for their purification.

The invention also relates to the discovery of an apparent functional relationship between HER4 and HER2, and the therapeutic aspects of the invention include those which are based on applicants' preliminary understanding of this relationship. Applicants' data strongly suggests that HER4 interacts with HER2 either by heterodimer formation or receptor crosstalk, and that such interaction appears to be one mechanism by which the HER4 receptor mediates effects on cell behavior. The reciprocal consequence is that HER2 activation is in some circumstances mediated through HER4.

In this connection, it appears that although heregulin induces phosphorylation of HER2 in cells expressing HER2 and HER4. Heregulin does not directly stimulate HER2 but acts by stimulating tyrosine phosphorylation of HER4.

Recognition of HER4 as a primary component of the heregulin signal transduction pathway opens a number of novel approaches to the diagnosis and treatment of human cancers in which the aberrant expression and/or function of heregulin and/or HER4 are involved. The therapeutic aspects of this invention thus include mediating a ligand's affect on HER4 and HER2 through antagonists, agonists or antibodies to HER4 ligands or HER4 receptor itself.

The invention also relates to chimeric proteins that specifically target and kill HER4 expressing tumor cells, polynucleotides encoding such chimeric proteins, and methods of using both in the therapeutic treatment of cancer and other human malignancies. Applicants' data demonstrate that such recombinant chimeric proteins specifically bind to the HER4 receptor and are cytotoxic against tumor cells that express HER4 on their surface. The bifunctional retention of both the specificity of the cell-binding portion of the molecule and the cytotoxic potential of the toxin portion makes for a very potent and targeted reagent.

The invention further relates to a method allowing determination of the cytotoxic activity of HER4 directed cytotoxic substances on cancer cells, thereby providing a powerful diagnostic tool; this will be of particular interest for prognosis of the effectiveness of these substances on an individual malignancy prior their therapeutic use.

4. Brief Description of the Figures

Figures 1/1 through 1/5. Nucleotide sequence [SEQ ID No:1] and deduced amino acid sequence of HER4 of the coding sequence from position 34 to 3961 (1308 amino acid residues) [SEQ ID No:2]. Nucleotides are numbered on the left, and amino acids are numbered above the sequence.

Figures 2/1 through 2/4. Nucleotide sequence [SEQ ID No:3] and deduced amino acid sequence ([SEQ ID No:4] of cDNAs encoding HER4 with alternate 3' end and without autophosphorylation domain. This sequence is identical with that of HER4 shown in Figures 1/1 through 1/5 up to nucleotide 3168, where the sequence diverges and the open reading frame stops after 13

amino acids, followed by an extended, unique 3'-untranslated region.

Figures 3/1 through 3/3. Nucleotide sequence [SEQ ID No:5] and deduced amino acid sequence [SEQ ID No:6] of cDNA encoding HER4 with a N-terminal truncation. This sequence contains the 3'-portion of the HER4 sequence where nucleotide position 156 of the truncated sequence aligns with position 2335 of the complete HER4 sequence shown in Figures 1/1 through 1/5 (just downstream from the region encoding the ATP-binding site of the HER4 kinase). The first 155 nucleotides of the truncated sequence are unique from HER4 and may represent the 5'-untranslated region of a transcript derived from a cryptic promoter within an intron of the HER4 gene. (Section 6.2.2., *infra*).

Figures 4/1, 4/2 and 5. The deduced amino acid sequence of two variant forms of human HER4 aligned with the full length HER4 receptor as represented in Figures 1/1 through 1/5. Sequences are displayed using the single-letter code and are numbered on the right with the complete HER4 sequence on top and the variant sequences below. Identical residues are indicated by a colon between the aligned residues.

Figures 4/1 and 4/2. HER4 with alternate 3'-end, lacking an autophosphorylation domain [SEQ ID No. 4]. This sequence is identical with that of HER4, shown in Figures 1/1 through 1/5, up to amino acid 1045, where the sequence diverges and continues for 13 amino acids before reaching a stop codon.

Figure 5. HER4 with N-terminal truncation [SEQ ID No. 6]. This sequence is identical to the 3'-portion of the HER4 shown in Figures 1/1 through 1/5 beginning at amino acid 768. (Section 6.2.2., *infra*).

Figures 6/1 and 6/2. Deduced amino acid sequence of human HER4 and alignment with other human EGFR-

family members (EGFR [SEQ ID No:7]; HER2 [SEQ ID No:8]; HER3 [SEQ ID No:9]). Sequences are displayed using the single-letter code and are numbered on the left. Identical residues are denoted with dots, gaps are introduced for optimal alignment, cysteine residues are marked with an asterisk, and N-linked glycosylation sites are denoted with a plus (+). Potential protein kinase C phosphorylation sites are indicated by arrows (HER4 amino acid positions 679, 685, and 699). The predicted ATP-binding site is shown with 4 circled crosses, C-terminal tyrosines are denoted with open triangles, and tyrosines in HER4 that are conserved with the major autophosphorylation sites in the EGFR are indicated with black triangles. The predicted extracellular domain extends from the boundary of the signal sequence marked by an arrow at position 25, to the hydrophobic transmembrane domain which is overlined from amino acid positions 650 through 675. Various subdomains are labeled on the right: I, II, III, and IV = extracellular subdomains (domains II and IV are cysteine-rich); TM = transmembrane domain; TK = tyrosine kinase domain. Domains I, III, TK are boxed.

Figure 7. Hydropathy profile of HER4, aligned with a comparison of protein domains for HER4 (1308 amino acids), EGFR (1210 amino acids), HER2 (1255 amino acids), and HER3 (1342 amino acids). The signal peptide is represented by a stippled box, the cysteine-rich extracellular subdomains are hatched, the transmembrane domain is filled, and the cytoplasmic tyrosine kinase domain is stippled. The percent amino acid sequence identities between HER4 and other EGFR-family members are indicated. Sig, signal peptide; I, II, III, and IV, extracellular domains; TM, transmembrane domain; JM, juxtamembrane

domain; CaIn, calcium influx and internalization domain; 3'UTR, 3' untranslated region.

Figures 8A and 8B. Northern blot analysis from human tissues hybridized to HER4 probes. RNA size markers (in kilobases) are shown on the left. Lanes 1 through 8 represent 2 μg of poly(A)+ mRNA from pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart, respectively. Figure 8A, Northern blot analysis of mRNA from human tissues hybridized to HER4 probes from the 3'-autophosphorylation domain; Figure 8B, Northern blot analysis from human tissues hybridized to HER4 probes from the 5'-extracellular domain (see Section 6.2.3., *infra*).

Figures 9A and 9B. Immunoblot analysis of recombinant HER4 stably expressed in CHO-KI cells, according to procedure outlined in Section 7.1.3, *infra*. Membrane preparations from CHO-KI cells expressing recombinant HER4 were separated on 7% SDS-polyacrylamide gels and transferred to nitrocellulose. In Figure 9A, blots were hybridized with a monoclonal antibody to the C-terminus of HER2 (Ab3, Oncogene Science, Uniondale, NY) that cross-reacts with HER4. In Figure 9B, blots were hybridized with a sheep antipeptide polyclonal antibody to a common epitope of HER2 and HER4. Lane 1, parental CHO-KI cells; lanes 2 - 4, CHO-KI/HER4 cell clones 6, 21, and 3, respectively. Note the 180 kDa HER4 protein and the 130 kDa cross-reactive species. The size in kilodaltons of prestained high molecular weight markers (BioRad, Richmond, CA) is shown on the left.

Figures 10A through 10D. Specific activation of HER4 tyrosine kinase by a breast cancer differentiation factor (see Section 8., *infra*). Four recombinant cell lines, each of which was engineered to overexpress a single member of EGFR-family of

tyrosine kinase receptors (EGFR, HER2, HER3, and HER4), were prepared according to the methods described in Sections 7.1.2 and 8.1., *infra*. Cells from each of the four recombinant cell lines were stimulated with various ligand preparations and assayed for receptor tyrosine phosphorylation using the assay described in Section 8.2., *infra*. Figure 10A, CHO/HER4 #3 cells; Figure 10B, CHO/HER2 cells; Figure 10C, NRHER5 cells; and Figure 10D, 293/HER3 cells. Cells stimulated with: lane 1, buffer control; lane 2, 100 ng/ml EGF; lane 3, 200 ng/ml amphiregulin; lane 4, 10 ml phenyl, column fraction 17 (Section 9, *infra*); lane 5, 10 μl phenyl column fraction 14 (Section 9., *infra*, and see description of Figure 11, below). The size (in kilodaltons) of the prestained molecular weight markers are labeled on the left of each panel. The phosphorylated receptor in each series migrates just below the 221 kDa marker. Bands at the bottom of the gels are extraneous and are due to the reaction of secondary antibodies with the antibodies used in the immunoprecipitation.

Figures 11A through 11F. Biological and biochemical properties of the MDA-MB-453-cell differentiation activity purified from the conditioned media of HepG2 cells (Section 9., *infra*). Figures 11A and 11B show induction of morphologic differentiation. Conditioned media from HepG2 cells was subjected to ammonium sulfate fractionation, followed by dialysis against PBS. Dilutions of this material were added to MDA-MB-453 monolayer at the indicated protein concentrations. Figure 11A, control; Figure 11B, 80 ng per well; Figure 11C, 2.0 μg per well; Figure 11D, Phenyl-5PW column elution profile monitored at 230 nm absorbance; Figure 11E, Stimulation of MDA-MB-

453 tyrosine autophosphorylation with the following ligand preparations: None (control with no factor added); TGF-α (50 ng/ml); CM (16-fold concentrated HepG2 conditioned medium tested at 2 μl and 10 μl per well); fraction (phenyl column fractions 13 to 20, 10 μl per well). Figure 11F, Densitometry analysis of the phosphorylation signals shown in Figure 11E.

Figures 12A and 12B. NDF-induced tyrosine phosphorylation. Figure 12A, MDA-MB-453 cells (lane 1, mock transfected COS cell supernatant; lane 2, NDF transfected COS cell supernatant); Figure 12B, CHO/HER4 21-2 cells (lanes 1 and 2, mock transfected COS cell supernatant; lanes 3 and 4, NDF transfected COS cell supernatant). See Section 10., *infra*. Tyrosine phosphorylation was determined by the tyrosine kinase stimulation assay described in Section 8.2., *infra*.

Figures 13A and 13B. Regional location of the HER4 gene to human chromosome 2 band q33. Figure 13A, Distribution of 124 sites of hybridization on human chromosomes; Figure 13B, Distribution of autoradiographic grains on diagram of chromosome 2.

Figure 14. Amino acid sequence of HER4-Ig fusion protein [SEQ ID No:10] (Section 5.4., *infra*).

Figure 15. Recombinant heregulin induces tyrosine phosphorylation of HER4. Tyrosine phosphorylated receptors were detected by Western blotting with an anti-phosphotyrosine Mab. Arrows indicate the HER2 and HER4 proteins. Monolayers of MDA-MB453 or CHO/HER4 cells were incubated with media from COS-1 cells transfected with a rat heregulin expression plasmid (HRG), or with a cDM8 vector control (-). The media was either applied directly (1x) or after concentrating 20-fold (20x, and vector control). Solubilized cells were immunoprecipitated with anti-phosphotyrosine Mab. Monolayers of CHO/HER2 cells were incubated as above with transfected Cos-1 cell supernatants or with two stimulatory Mabs to HER2

(Mab 28 and 29). Solubilized cells were immunoprecipitated with anti-HER2 Mab.

Figures 16A through 16C. Expression of recombinant HER2 and HER4 in human CEM cells. Transfected CEM cells were selected that stably express either HER2, HER4, or both recombinant receptors. In Figure 16A, recombinant HER2 was detected by immunmoprecipitation of cell lysates with anti-HER2 Mab (Ab-2) and Western blotting with another anti-HER2 Mab (Ab-3). In Figure 16B, Recombinant HER4 was detected by immunoprecipitation of ^{35}S-labeled cell lysates with HER4-specific rabbit anti-peptide antisera. In Figure 16C, Three CEM cell lines were selected that express one or both recombinant receptors and aliquots of each were incubated with media control (-), with two HER2-stimulatory Mabs (Mab 28 and 29), or with an isotype matched control Mab (18.4). Solubilized cells were immunoprecipitated with anti-HER2 Mab (Ab-2) and tyrosine phosphorylated HER2 was detected by Western blotting with an anti-phosphotyrosine Mab. The size in kilodaltons of prestained high molecular weight markers (Bio-Rad) is shown on the left and arrows indicate the HER2 and HER4 proteins.

Figures 17A through 17C. Heregulin induces tyrosine phosphorylation in CEM cells expressing HER4. Three CEM cell lines that express either HER2 or HER4 alone (CEM 1-3 and CEM 3-13) or together (CEM 2-9) were incubated with 7x concentrated supernatants from mock-(-) or heregulin-transfected (+) COS-1 cells. Solubilized cells were immunoprecipitated (IP) with anti-phosphotyrosine Mab (PY20); in Figure 17A,

HER2-specific anti-HER2 Mab (Ab-2); in Figure 17B, HER4-specific Mab (6-4); in Figure 17C, in each case tyrosine phosphorylated receptors were detected by Western blotting with anti-phosphotyrosine Mab. The size in kilodaltons of prestained molecular weight markers (BioRad) is shown on the left and arrows indicate the HER2 and HER4 proteins. HRG, recombinant rat heregulin.

Figure 18. Covalent cross-linking of iodinated heregulin to HER4. ^{125}I-heregulin was added to CHO/HER4 or CHO/HER2 cells for 2 h at 4° C. Washed cells were cross-linked with BS^3, lysed, and the proteins separated using 7% PAGE. Labeled bands were detected on the phosphorimager. Molecular weight markers are shown on the left.

Figures 19A through 19D. Purification of p45 from HepG2 conditioned media. Column fractions were tested for their potential to induce differentiation of MDA-MB-453 cells. Active fractions were pooled as indicated by an horizontal bar. Figure 19A, Concentrated HepG2 conditioned medium was subjected to 50% ammonium sulfate precipitation. Supernatant resulting from this step was subjected to hydrophobic interaction chromatography using phenyl-Sepharose. Pooled fractions were then loaded on a DEAE-Sepharose column. Figure 19B, the DEAE-Sepharose column flow-through was subjected to CM-Sepharose chromatography. Figure 19C, Affinity Chromatography of the MDA-MB-453 differentiation factor using heparin-5PW column. Fractions 35-38 eluting around 1.3M NaCl were pooled. Figure 19D, Size Exclusion chromatography of the differentiation factor. The molecular masses of calibration standards are indicated in kilodaltons.

Figure 20. Aliquots (25 microliter) of the active size exclusion column fractions (30 and 32) were electrophoresed under reducing conditions on a 12.5% polyacrylamide gel. The gel was silver-stained.

Molecular masses of Bio-Rad silver stain standards are indicated in kilodaltons.

Figures 21A through 21C. Stimulation of tyrosine phosphorylation by p45. Figure 21A, Size exclusion column fractions were tested on MDA-MB-453 cells for the induction of tyrosine phosphorylation. Cell lysates were then electrophoresed on a 4-15% polyacrylamide gel. After transfer to nitrocellulose, proteins were probed with a phosphotyrosine antibody and phosphoproteins detected by chemiluminescence. The molecular mass of the predominantly phosphorylated protein is indicated. Figure 21B, the experiments were performed on cells that had been transfected with expression plasmids for either HER4 (CHO/HER4) or HER2 (CHO/HER2). Cell monolayers were incubated in the absence or the presence of p45 (size exclusion column fraction 32, 100 ng/ml). Samples were then processed as indicated in Figure 21A except that a 7.5% polyacrylamide gel was used to separate the CHO/HER2 cell lysates. Figure 21C, CHO/HER2 cells were incubated in the presence or the absence of N29 monoclonal antibody to the extracellular domain of $p185^{erbB2}$. Cell lysates were immunoprecipitated with the Ab-3 monoclonal antibody to $p185^{erbB2}$. Precipitated proteins were subjected to SDS-PAGE, and phosphoproteins were detected as indicated under Section 13.4., *supra*.

Figures 22A and 22B. Binding and cross-linking of ^{125}I-p45 to CHO-KI, CHO-HER2 and CHO/HER4 cells. Figure 22A, Scatchard analysis of the binding of ^{125}I-p45 to CHO/HER4 cells. Increasing concentrations of ^{125}I-p45 were incubated with cell monolayers for 2 h at 4° C. Nonspecific binding was subtracted from all cell-associated radioactivity data values. A Scatchard plot as well as a saturation curve of the binding data are shown. Figure 22B, Covalent cross-linking. ^{125}I-p45 was added to the cells in the presence or absence of an excess of unlabeled p45 for

2 h at 4° C. After washing of the cells to remove unbound iodinated material, the cross-linking reagent bis-(sulfosuccinimidyl)-suberate was added to the cells for 45 min. at 4° C. Cells were lysed and proteins separated by electrophoresis on a 7.5% polyacrylamide gel. Molecular masses of protein standards are indicated in kilodaltons. A Molecular Dynamics PhosphoImager was used to visualize the radioactive species.

Figures 23A and 23B. Construction of the HAR-TX β2 expression plasmid, encoding the hydrophilic leader sequence of amphiregulin (AR), heregulin β2, and PE40, under control of the IPTG inducible T7 promoter; Figure 23A, schematic diagram of the expression plasmid pSE 8.4, encoding HAR-TX β2; Figure 23B, amino acid sequence of HAR β2, the ligand portion of HAR-TX β2, composed of the AR leader sequence and rat heregulin β2 [SEQ ID No:40].

Figures 24A and 24B. cDNA sequence [SEQ ID No:41] and deduced amino acid sequence [SEQ ID No:42] of the chimera HAR-TX β2, comprising the amphiregulin (AR) leader sequence and the coding sequences of rat heregulin *Pseudomonas* exotoxin PE40. The linker sequence between the two portions is indicated by a bar above the sequence, the ligand portion is located at the 5' (N-terminal), the PE40 exotoxin portion is located at the 3' (C-terminal) part of the sequence. Nucleotides are numbered on the right side, and amino acids are numbered below the sequence.

Figure 25. Purification of the chimeric HAR-TX b2 protein: shown is a Coomassie brilliant blue stained SDS-PAGE (4-20%) of the different purification steps. Lanes 1 - 5 have been loaded under reducing conditions. Lane 1, MW standards; lane 2, refolded HAR-TX β2, 20x concentrated; lane 3, POROS HS flow-through, 20x concentrated; lane 4, POROS HS eluate;

lane 5, Source 15S eluate (pure HAR-TX β2, 2 μg); lane 6, 2 μg HAR-TX β2, loaded under non-reducing conditions.

Figure 26. Membrane-based ELISA binding analysis, performed to determine the binding activity of the purified HAR-TX β2 protein. Binding of HAR-TX β2 (O) and PE40 (●) to membranes prepared from the HER4 expressing human breast carcinoma cell line.

Figure 27. HAR-TX bβ2 induced tyrosine phosphorylation in transfected CEM cells. CEM cells co-expressing HER4 and HER2 (H2,4), or expressing HER4 (H4), HER2 (H2), HER1 (H1) alone, respectively, were incubated in the presence (+) or absence (-) of HAR-TX β2, then solubilized, and immunoblotted with the monoclonal anti-phosphotyrosine antibody PY20. The arrow indicates the phosphorylated receptor band, the molecular weight is indicated in kDA.

Figures 28A and 28B. Cytotoxic effect of HAR-TX β2 on tumor cell lines. Figure 28A, following 48 hours incubation with HAR-TX β2, the cell killing effect of HAR-TX β2 on the tumor cell lines LNCaP (■), AU565 (O), SKBR3 (●), and SKOV3 (□) by quantification of fluorescent calcein cleaved from calcein-AM. Figure 28B, Competitive cytotoxicity of HAR-TX β2 with heregulin β2-Ig. LNCaP cells were co-incubated with 50 ng/ml HAR-TX β2 and increasing concentrations (2-5000 ng/ml) of either heregulin β2-Ig (□) or L6-Ig (■). The data represent the mean of triplicate assays.

Figure 29. HAR-TX β2 induced tyrosine phosphorylation in tumor cells expressing HER3 (L2987) or co-expressing HER2 and HER3 (H3396). Cells were incubated in the presence (+) or in the absence (-) of HAR-TX β2, solubilized, and immunoblotted with the monoclonal anti-phosphotyrosine antibody PY20.

Phosphorylated receptors are indicated by an arrow, the molecular weight is indicated in kDa.

5. Detailed Description of the Invention

The present invention is directed to HER4/p180^{erbB4} ("HER4"), a closely related yet distinct member of the Human EGF Receptor (HER)/neu subfamily of receptor tyrosine kinases, as well as HER4-encoding polynucleotides (e.g., cDNAs, genomic DNAs, RNAs, anti-sense RNAs, etc.), the production of mature and precursor forms of HER4 from a HER4 polynucleotide coding sequence, recombinant HER4 expression vectors, HER4 analogues and derivatives, anti-HER4 antibodies, HER4 ligands, and diagnostic and therapeutic uses of HER4 polynucleotides, polypeptides, ligands, and antibodies in the field of human oncology and neurobiology.

As discussed in Section 2, *supra*, HER2 has been reported to be associated with a wide variety of human malignancies, thus the understanding of its activation mechanisms as well as the identification of molecules involved are of particular clinical interest. This invention uncovers an apparent functional relationship between the HER4 and HER2 receptors involving HER4-mediated phosphorylation of HER2, potentially via intracellular receptor crosstalk or receptor dimerization. In this connection, the invention also

provides HER4 ligands capable of inducing cellular differentiation in breast carcinoma cells that appears to involve HER4-mediated phosphorylation of HER2. Furthermore, applicants' data provide evidence that heregulin mediates biological effects on such cells not directly through HER2, as has been reported (Peles *et al.*, 1992, Cell 69:205-216), but instead by means of a direct interaction with HER4, and/or through an interaction with a HER2/ HER4 complex. In cell lines expressing both HER2 and HER4, binding of heregulin to HER4 may stimulate HER2 either by heterodimer formation of these two related receptors or by intracellular receptor crosstalk.

Recently, also HER3 has been reported to bind heregulin (see Section 2, *supra*). However, various observations indicate that the heregulin-mediated activation of HER3 varies considerably, depending on the context of expression, suggesting that other cellular components may be involved in the modulation of HER3 activity (reviewed in: Carraway and Cantley, 1994, Cell 78:5-8).

Unless otherwise indicated, the practice of the present invention utilizes standard techniques of molecular biology and molecular cloning, microbiology, immunology, and recombinant DNA known in the art. Such techniques are described and explained throughout the literature, and can be found in a number of more comprehensive publications such as, for example, Sambrook *et al.*, Molecular Cloning; A Laboratory Manual (Second Edition, 1989).

5.1. HER4 Polynucleotides

One aspect of the present invention is directed to HER4 polynucleotides, including recombinant polynucleotides encoding the prototype HER4

polypeptide shown in FIG. 1A and 1B, polynucleotides which are related or are complementary thereto, and recombinant vectors and cell lines incorporating such recombinant polynucleotides. The term "recombinant polynucleotide" as used herein refers to a polynucleotide of genomic, cDNA, synthetic or semisynthetic origin which, by virtue of its origin or manipulation, is not associated with any portion of the polynucleotide with which it is associated in nature, and may be linked to a polynucleotide other than that to which it is linked in nature, and includes single or double stranded polymers of ribonucleotides, deoxyribonucleotides, nucleotide analogs, or combinations thereof. The term also includes various modifications known in the art, including but not limited to radioactive and chemical labels, methylation, caps, internucleotide modifications such as those with charged linkages (*e.g.*, phosphorothothioates, phosphorodithothioates, etc.) and uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidites, carbamites, etc.), as well as those containing pendant moeties, intercalcators, chelators, alkylators, etc. Related polynucleotides are those having a contiguous stretch of about 200 or more nucleotides and sharing at least about 80% homology to a corresponding sequence of nucleotides within the nucleotide sequence disclosed in FIG. 1A and 1B. Several particular embodiments of such HER4 polynucleotides and vectors are provided in example Sections 6 and 7, *infra*.

HER4 polynucleotides may be obtained using a variety of general techniques known in the art, including molecular cloning and chemical synthetic methods. One method by which the molecular cloning of cDNAs encoding the prototype HER4 polypeptide of the

invention (FIG. 1A and 1 B), as well as several HER4 polypeptide variants, is described by way of example in Section 6., *infra*. Conserved regions of the sequences of EGFR, HER2, HER3, and Xmrk are used for selection of the degenerate oligonucleotide primers which are then used to isolate HER4. Since many of these sequences have extended regions of amino acid identity, it is difficult to determine if a short PCR fragment represents a unique molecule or merely the species-specific counterpart of EGFR, HER2, or HER3. Often the species differences for one protein are as great as the differences within species for two distinct proteins. For example, fish Xmrk has regions of 47/55 (85%) amino acid identity to human EGFR, suggesting it might be the fish EGFR, however isolation of another clone that has an amino acid sequence identical to Xmrk in this region (57/57) shows a much higher homology to human EGFR in its flanking sequence (92% amino acid homology) thereby suggesting that it, and not Xmrk, is the fish EGFR (Wittbrodt *et al.*, 1989, Nature 342:415-421). As described in Section 6., *infra*, it was necessary to confirm that a murine HER4/*erb*B4 PCR fragment was indeed a unique gene, and not the murine homolog of EGFR, HER2, or HER3, by isolating genomic fragments corresponding to murine EGFR, *erb*B2 and *erb*B3. Sequence analysis of these clones confirmed that this fragment was a novel member of the EGFR family. Notably a region of the murine clone had a stretch of 60/64 amino acid identity to human HER2, but comparison with the amino acid and DNA sequences of the other EGFR homologs from the same species (mouse) firmly established it encoded a novel transcript.

HER4 polynucleotides may be obtained from a variety of cell sources which produce HER4-like

activities and/or which express HER4-encoding mRNA. In this connection, applicants have identified a number of suitable human cell sources for HER4 polynucleotides, including but not limited to brain, cerebellum, pituitary, heart, skeletal muscle, and a variety of breast carcinoma cell lines (see Section 6., *infra*).

For example, polynucleotides encoding HER4 polypeptides may be obtained by cDNA cloning from RNA isolated and purified from such cell sources or by genomic cloning. Either cDNA or genomic libraries of clones may be prepared using techniques well known in the art and may be screened for particular HER4-encoding DNAs with nucleotide probes which are substantially complementary to any portion of the HER4 gene. Various PCR cloning techniques may also be used to obtain the HER4 polynucleotides of the invention. A number of PCR cloning protocols suitable for the isolation of HER4 polynucleotides have been reported in the literature (see, for example, PCR protocols: A Guide to Methods and Applications, Eds. Inis *et al.*, Academic Press, 1990).

For the construction of expression vectors, polynucleotides containing the entire coding region of the desired HER4 may be isolated as full length clones or prepared by splicing two or more polynucleotides together. Alternatively, HER4-encoding DNAs may be synthesized in whole or in part by chemical synthesis using techniques standard in the art. Due to the inherent degeneracy of nucleotide coding sequences, any polynucleotide encoding the desired HER4 polypeptide may be used for recombinant expression. Thus, for example, the nucleotide sequence encoding the prototype HER4 of the invention provided in FIG.

1A and 1B may be altered by substituting nucleotides such that the same HER4 product is obtained.

The invention also provides a number of useful applications of the HER4 polynucleotides of the invention, including but not limited to their use in the preparation of HER4 expression vectors, primers and probes to detect and/or clone HER4, and diagnostic reagents. Diagnostics based upon HER4 polynucleotides include various hybridization and PCR assays known in the art, utilizing HER4 polynucleotides as primers or probes, as appropriate. One particular aspect of the invention relates to a PCR kit comprising a pair of primers capable of priming cDNA synthesis in a PCR reaction, wherein each of the primers is a HER4 polynucleotide of the invention. Such a kit may be useful in the diagnosis of certain human cancers which are characterized by aberrant HER4 expression. For example, certain human carcinomas may overexpress HER4 relative to their normal cell counterparts, such as human carcinomas of the breast. Thus, detection of HER4 overexpression mRNA in breast tissue may be an indication of neoplasia. In another, related embodiment, human carcinomas characterized by overexpression of HER2 and expression or overexpression of HER4 may be diagnosed by a polynucleotide-based assay kit capable of detecting both HER2 and HER4 mRNAs, such a kit comprising, for example, a set of PCR primer pairs derived from divergent sequences in the HER2 and HER4 genes, respectively.

5.2. HER4 Polypeptides

Another aspect of the invention is directed to HER4 polypeptides, including the prototype HER4 polypeptide provided herein, as well as polypeptides

derived from or having substantial homology to the amino acid sequence of the prototype HER4 molecule. The term "polypeptide" in this context refers to a polypeptide prepared by synthetic or recombinant means, or which is isolated from natural sources. The term "substantially homologous" in this context refers to polypeptides of about 80 or more amino acids sharing greater than about 90% amino acid homology to a corresponding contiguous amino acid sequence in the prototype HER4 primary structure (FIG. 1A and 1B). The term "prototype HER4" refers to a polypeptide having the amino acid sequence of precursor or mature HER4 as provided in FIG. 1A and 1B, which is encoded by the consensus cDNA nucleotide sequence also provided therein, or by any polynucleotide sequence which encodes the same amino acid sequence.

HER4 polypeptides of the invention may contain deletions, additions or substitutions of amino acid residues relative to the sequence of the prototype HER4 depicted in FIG. 1A and 1B which result in silent changes thus producing a bioactive product. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the resides involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

The HER4 polypeptide depicted in FIG. 1A and 1B has all of the fundamental structural features

characterizing the EGFR-family of receptor tyrosine kinases (Hanks *et al.*, 1988, Science 241:42-52). The precursor contains a single hydrophobic stretch of 26 amino acids characteristic of a transmembrane region that bisects the protein into a 625 amino acid extracellular ligand binding domain, and a 633 amino acid C-terminal cytoplasmic domain. The ligand binding domain can be further divided into 4 subdomains (I - IV), including two cysteine-rich regions (II, residues 186-334; and IV, residues 496-633), and two flanking domains (I, residues 29-185; and III, residues 335-495) that may define specificity for ligand binding (Lax *et al.*, 1988, Mol. Cell. Biol. 8:1970-78). The extracellular domain of HER4 is most similar to HER3, where domains II-IV of HER4 share 56-67% identity to the respective domains of HER3. In contrast, the same regions of EGFR and HER2 exhibit 43-51% and 34-46% homology to HER4, respectively (FIG. 6A and 6B). The 4 extracellular subdomains of EGFR and HER2 share 39-50% identity. HER4 also conserves all 50 cysteines present in the extracellular portion of EGFR, HER2, and HER3, except that the HER2 protein lacks the fourth cysteine in domain IV. There are 11 potential N-linked glycosylation sites in HER4, conserving 4 of 12 potential sites in EGFR, 3 of 8 sites in HER2, and 4 of 10 sites in HER3.

Following the transmembrane domain of HER4 is a cytoplasmic juxtamembrane region of 37 amino acids. This region shares the highest degree of homology with EGFR (73% amino acid identity) and contains two consensus protein kinase C phosphorylation sites at amino acid residue numbers 679 (Serine) and 699 (Threonine) in the FIG. 1A and 1B sequence, the latter of which is present in EGFR and HER2. Notably, HER4 lacks a site analogous to Thr654 of EGFR.

Phosphorylation of this residue in the EGFR appears to block ligand-induced internalization and plays an important role in its transmembrane signaling (Livneh *et al.*, 1988, Mol. Cell. Biol. 8:2302-08). HER4 also contains Thr692 analogous to Thr694 of HER2. This threonine is absent in EGFR and HER3 and has been proposed to impart cell-type specificity to the mitogenic and transforming activity of the HER2 kinase (DiFiore *et al.* 1992, EMBO J. 11:3927-33). The juxtamembrane region of HER4 also contains a MAP kinase consensus phosphorylation site at amino acid number 699 (Threonine), in a position homologous to Thr699 of EGFR which is phosphorylated by MAP kinase in response to EGF stimulation (Takishima *et al.*, 1991, Proc. Natl. Acad. Sci. U.S.A. 88:2520-25).

The remaining cytoplasmic portion of HER4 consists of a 276 amino acid tyrosine kinase domain, an acidic helical structure of 38 amino acids that is homologous to a domain required for ligand-induced internalization of the EGFR (Chen *et al.*, 1989, Cell 59:33-43), and a 282 amino acid region containing 18 tyrosine residues characteristic of the autophosphorylation domains of other EGFR-related proteins (FIG. 6A and 6B). The 276 amino acid tyrosine kinase domain conserves all the diagnostic structural motifs of a tyrosine kinase, and is most related to the catalytic domains of EGFR (79% identity) and HER2 (77% identity), and to a lesser degree, HER3 (63% identity). In this same region, EGFR and HER2 share 83% identity. Examples of the various conserved structural motifs include the following: the ATP-binding motif (GXGXXG) [SEQ ID No:11] with a distal lysine residue that is predicted to be involved in the phosphotransfer reaction (Hanks *et al.*, 198, Science 241:42-52; Hunter and Cooper, in

The Enzymes Vol. 17 (eds. Boyer and Krebs) pp. 191-246 (Academic Press 1986)); tyrosine-kinase specific signature sequences (DLAARN [SEQ ID No:12] and PIKWMA [SEQ ID No:13]) and Tyr875 (FIG. 6A and 6B), a residue that frequently serves as an autophosphorylation site in many tyrosine kinases (Hunter and Cooper, *supra*); and approximately 15 residues that are either highly or completely conserved among all known protein kinases (Plowman *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:4905-09; Hanks *et al.*, *supra*). The C-terminal 282 amino acids of HER4 has limited homology with HER2 (27%) and EGFR (19%). However, the C-terminal domain of each EGFR-family receptor is proline-rich and conserves stretches of 2-7 amino acids that are generally centered around a tyrosine residue. These residues include the major tyrosine autophosphorylation sites of EGFR at Tyr1068, Tyr1086, Tyr1148, and Tyr1173 (FIG. 6A and 6B, filled triangles; Margolis *et al.*, 1989, J. Biol. Chem. 264:10667-71).

5.3. Recombinant Synthesis of HER4 Polypeptides

The HER4 polypeptides of the invention may be produced by the cloning and expression of DNA encoding the desired HER4 polypeptide. Such DNA may be ligated into a number of expression vectors well known in the art and suitable for use in a number of acceptable host organisms, in fused or mature form, and may contain a signal sequence to permit secretion. Both prokaryotic and eukaryotic host expression systems may be employed in the production of recombinant HER4 polypeptides. For example, the prototype HER4 precursor coding sequence or its functional equivalent may be used in a host cell capable of processing the precursor correctly. Alternatively, the coding

sequence for mature HER4 may be used to directly express the mature HER4 molecule. Functional equivalents of the HER4 precursor coding sequence include any DNA sequence which, when expressed inside the appropriate host cell, is capable of directing the synthesis, processing and/or export of HER4.

Production of a HER4 polypeptide using recombinant DNA technology may be divided into a four-step process for the purposes of description: (1) isolation or generation of DNA encoding the desired HER4 polypeptide; (2) construction of an expression vector capable of directing the synthesis of the desired HER4 polypeptide; (3) transfection or transformation of appropriate host cells capable of replicating and expressing the HER4 coding sequence and/or processing the initial product to produce the desired HER4 polypeptide; and (4) identification and purification of the desired HER4 product.

5.3.1. Isolation or Generation of HER4 Encoding DNA

HER4-encoding DNA, or functional equivalents thereof, may be used to construct recombinant expression vectors which will direct the expression of the desired HER4 polypeptide product. In a specific embodiment, DNA encoding the prototype HER4 polypeptide (FIG. 1A and 1B), or fragments or functional equivalents thereof, may be used to generate the recombinant molecules which will direct the expression of the recombinant HER4 product in appropriate host cells. HER4-encoding nucleotide sequences may be obtained from a variety of cell sources which produce HER4-like activities and/or which express HER4-encoding mRNA. For example, HER4-encoding cDNAs may be obtained from the breast adenocarcinoma cell line MDA-MB-453 (ATCC HTB131) as

described in Section 6., *infra*. In addition, a number of human cell sources are suitable for obtaining HER4 cDNAs, including but not limited to various epidermoid and breast carcinoma cells, and normal heart, kidney, and brain cells (see Section 6.2.3., *infra*).

The HER4 coding sequence may be obtained by molecular cloning from RNA isolated and purified from such cell sources or by genomic cloning. Either cDNA or genomic libraries of clones may be prepared using techniques well known in the art and may be screened for particular HER4-encoding DNAs with nucleotide probes which are substantially complementary to any portion of the HER4 gene. Alternatively, cDNA or genomic DNA may be used as templates for PCR cloning with suitable oligonucleotide primers. Full length clones, *i.e.*, those containing the entire coding region of the desired HER4 may be selected for constructing expression vectors, or overlapping cDNAs can be ligated together to form a complete coding sequence. Alternatively, HER4-encoding DNAs may be synthesized in whole or in part by chemical synthesis using techniques standard in the art.

5.3.2. Construction of HER4 Expression Vectors

Various expression vector/host systems may be utilized equally well by those skilled in the art for the recombinant expression of HER4 polypeptides. Such systems include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the desired HER4 coding sequence; yeast transformed with recombinant yeast expression vectors containing the desired HER4 coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*,

baculovirus) containing the desired HER4 coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the desired HER4 coding sequence; or animal cell systems infected with recombinant virus expression vectors (*e.g.*, adenovirus, vaccinia virus) including cell lines engineered to contain multiple copies of the HER4 DNA either stably amplified (*e.g.*, CHO/dhfr, CHO/glutamine synthetase) or unstably amplified in double-minute chromosomes (*e.g.*, murine cell lines).

The expression elements of these vectors vary in their strength and specificities. Depending on the host/vector system utilized, any one of a number of suitable transcription and translation elements may be used. For instance, when cloning in mammalian cell systems, promoters isolated from the genome of mammalian cells, (*e.g.*, mouse metallothionein promoter) or from viruses that grow in these cells, (*e.g.*, vaccinia virus 7.5K promoter or Moloney murine sarcoma virus long terminal repeat) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted sequences.

Specific initiation signals are also required for sufficient translation of inserted protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire HER4 gene including its own initiation codon and adjacent sequences are inserted into the appropriate expression vectors, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence

is inserted, exogenous translational control signals, including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the HER4 coding sequences to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of transcription attenuation sequences, enhancer elements, etc.

For example, in cases where an adenovirus is used as a vector for driving expression in infected cells, the desired HER4 coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E3 or E4) will result in a recombinant virus that is viable and capable of expressing HER4 in infected hosts. Similarly, the vaccinia 7.5K promoter may be used. An alternative expression system which could be used to express HER4 is an insect system. In one such system, Autographa californica nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The HER4 coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the HER4 coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i*.e., virus lacking the proteinaceous coat encoded by the polyhedrin gene).

These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. Yet another approach uses retroviral vectors prepared in amphotropic packaging cell lines, which permit high efficiency expression in numerous cells types. This method allows one to assess cell-type specific processing, regulation or function of the inserted protein coding sequence.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers (*e.g.*, zinc and cadmium ions for metallothionein promoters). Therefore, expression of the recombinant HER4 polypeptide may be controlled. This is important if the protein product of the cloned foreign gene is lethal to host cells. Furthermore, modifications (*e.g.*, phosphorylation) and processing (*e.g.*, cleavage) of protein products are important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of protein. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

5.3.3. Transformants Expressing HER4 Gene Products

The host cells which contain the recombinant coding sequence and which express the desired HER4 polypeptide product may be identified by at least four general approaches (a) DNA-DNA, DNA-RNA or RNA-antisense RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression

of HER4 mRNA transcripts in the host cell; and (d) detection of the HER4 product as measured by immunoassay and, ultimately, by its biological activities.

In the first approach, for example, the presence of HER4 coding sequences inserted into expression vectors can be detected by DNA-DNA hybridization using hybridization probes and/or primers for PCR reactions comprising polynucleotides that are homologous to the HER4 coding sequence.

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, resistance to methotrexate (MTX), resistance to methionine sulfoximine (MSX), transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the HER4 coding sequence is inserted within a marker gene sequence of the vector, recombinants containing that coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the HER4 sequence under the control of the same or different promoter used to control the expression of the HER4 coding sequence. Expression of the marker in response to induction or selection indicates expression of the HER4 coding sequence. In a particular embodiment described by way of example herein, a HER4 expression vector incorporating glutamine synthetase as a selectable marker is constructed, used to transfect CHO cells, and amplified expression of HER4 in CHO cells is obtained by selection with increasing concentration of MSX.

In the third approach, transcriptional activity for the HER4 coding region can be assessed by hybridization assays. For example, polyadenylated RNA can be isolated and analyzed by Northern blot using a probe homologous to the HER4 coding sequence or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of HER4 can be assessed immunologically, for example by Western blots, immunoassays such as radioimmunoprecipitation, enzyme-linked immunoassays and the like. Alternatively, expression of HER4 may be assessed by detecting a biologically active product. Where the host cell secretes the gene product the cell free media obtained from the cultured transfectant host cell may be assayed for HER4 activity. Where the gene product is not secreted, cell lysates may be assayed for such activity. In either case, assays which measure ligand binding to HER4, HER4 phosphorylation, or other bioactivities of HER4 may be used.

5.4. Anti-HER4 Antibodies

The invention is also directed to polyclonal and monoclonal antibodies which recognize epitopes of HER4 polypeptides. Anti-HER4 antibodies are expected to have a variety of useful applications in the field of oncology, several of which are described generally below. More detailed and specific descriptions of various uses for anti-HER4 antibodies are provided in the sections and subsections which follow. Briefly, anti-HER4 antibodies may be used for the detection and quantification of HER4 polypeptide expression in cultured cells, tissue samples, and *in vivo*. Such

immunological detection of HER4 may be used, for example, to identify, monitor, and assist in the prognosis of neoplasms characterized by aberrant or attenuated HER4 expression and/or function. Additionally, monoclonal antibodies recognizing epitopes from different parts of the HER4 structure may be used to detect and/or distinguish between native HER4 and various subcomponent and/or mutant forms of the molecule. Anti-HER4 antibody preparations are also envisioned as useful biomodulatory agents capable of effectively treating particular human cancers. In addition to the various diagnostic and therapeutic utilities of anti-HER4 antibodies, a number of industrial and research applications will be obvious to those skilled in the art, including, for example, the use of anti-HER4 antibodies as affinity reagents for the purification of HER4 polypeptides, and as immunological probes for elucidating the biosynthesis, metabolism and biological functions of HER4.

Anti-HER4 antibodies may be useful for influencing cell functions and behaviors which are directly or indirectly mediated by HER4. As an example, modulation of HER4 biological activity with anti-HER4 antibodies may influence HER2 activation and, as a consequence, modulate intracellular signals generated by HER2. In this regard, anti-HER4 antibodies may be useful to effectively block ligand-induced, HER4-mediated activation of HER2, thereby affecting HER2 biological activity. Conversely, anti-HER4 antibodies capable of acting as HER4 ligands may be used to trigger HER4 biological activity and/or initiate a ligand-induced, HER4-mediated effect on HER2 biological activity, resulting in a cellular

response such as differentiation, growth inhibition, etc.

Additionally, anti-HER4 antibodies conjugated to cytotoxic compounds may be used to selectively target such compounds to tumor cells expressing HER4, resulting in tumor cell death and reduction or eradication of the tumor. In a particular embodiment, toxin-conjugated antibodies having the capacity to bind to HER4 and internalize into such cells are administered systemically for targeted cytotoxic effect. The preparation and use of radionuclide and toxin conjugated anti-HER4 antibodies are further described in Section 5.5., *infra*.

Overexpression of HER2 is associated with several human cancers. Applicants' data indicate that HER4 is expressed in certain human carcinomas in which HER2 overexpression is present. Therefore, anti-HER4 antibodies may have growth and differentiation regulatory effects on cells which overexpress HER2 in combination with HER4 expression, including but not limited to breast adenocarcinoma cells. Accordingly, this invention includes antibodies capable of binding to the HER4 receptor and modulating HER2 or HER2-HER4 functionality, thereby affecting a response in the target cell. For the treatment of cancers involving HER4-mediated regulation of HER2 biological activity, agents capable of selectively and specifically affecting the intracellular molecular interaction between these two receptors may be conjugated to internalizing anti-HER4 antibodies. The specificity of such agents may result in biological effects only in cells which co-express HER2 and HER4, such as breast cancer cells.

Various procedures known in the art may be used for the production of polyclonal antibodies to

epitopes of HER4. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of anti-HER4 antibodies by immunization with one or more injections of a HER4 polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

A monoclonal antibody to an epitope of HER4 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 495-497), and the more recent human B-cell hybridoma technique (Kosbor *et al.*, 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.*, 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.*, 1984, Nature, 312:604-608; Takeda *et al.*, 1985, Nature, 314:452-454). Alternatively, techniques described for the production of single chain

antibodies (U.S. Patent 4,946,778) can be adapted to produce HER4-specific single chain antibodies. Recombinant human or humanized versions of anti-HER4 monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (*e.g.*, Jones *et al.*, 1986, Nature 321:522-25; Reichman *et al.*, 1988, Nature 332:323-27; Verhoeyen *et al.*, 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized anti-HER2 monoclonal antibody may also be employed in the production of humanized anti-HER4 antibodies (Carter *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating a recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant anti-HER4 antibodies (*e.g.*, Huse *et al.*, 1989, Science 246:1275-81).

As an example, anti-HER4 monoclonal antibodies may be generated by immunization of mice with cells selectively overexpressing HER4 (*e.g.*, CHO/HER4 21-2 cells as deposited with the ATCC) or with partially purified recombinant HER4 polypeptides. In one embodiment, the full length HER4 polypeptide (FIG. 1A and 1B) may be expressed in Baculovirus systems, and membrane fractions of the recombinant cells used to immunize mice. Hybridomas are then screened on CHO/HER4 cells (*e.g.*, CHO HER4 21-2 cells as deposited with the ATCC) to identify monoclonal antibodies reactive with the extracellular domain of HER4. Such monoclonal antibodies may be evaluated for their ability to block NDF, or HepG2-differentiating factor, binding to HER4; for their ability to bind and stay resident on the cell surface, or to internalize into

cells expressing HER4; and for their ability to directly upregulate or downregulate HER4 tyrosine autophosphorylation and/or to directly induce a HER4-mediated signal resulting in modulation of cell growth or differentiation. In this connection, monoclonal antibodies N28 and N29, directed to HER2, specifically bind HER2 with high affinity. However, monoclonal N29 binding results in receptor internalization and downregulation, morphologic differentiation, and inhibition of HER2 expressing tumor cells in athymic mice. In contrast, monoclonal N28 binding to HER2 expressing cells results in stimulation of autophosphorylation, and an acceleration of tumor cell growth both *in vitro* and *in vivo* (Bacus *et al.*, 1992, Cancer Res. 52:2580-89; Stancovski *et al.*, 1991, Proc. Natl. Acad. Sci. U.S.A. 88:8691-95). In yet another embodiment, a soluble recombinant HER4-Immunoglobulin (HER4-Ig) fusion protein is expressed and purified on a Protein A affinity column. The amino acid sequence of one such HER4-Ig fusion protein is provided in FIG. 14. The soluble HER4-Ig fusion protein may then be used to screen phage libraries designed so that all available combinations of a variable domain of the antibody binding site are presented on the surfaces of the phages in the library. Recombinant anti-HER4 antibodies may be propagated from phage which specifically recognize the HER4-Ig fusion protein.

Antibody fragments which contain the idiotype of the molecule may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab)'E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the two Fab fragments which can be

generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.*, 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to HER4 protein.

5.5. HER4 Ligands

One aspect of the present invention is directed to HER4 ligands. As defined herein, HER4 ligands are capable of binding to the 180K transmembrane protein, HER4/p180^{erbB4} or functional analogues thereof, and activating tyrosine kinase activity. Functional analogues of HER4/p180^{erbB4}-ligands are capable of activating HER4 tyrosine kinase activity. Activation of the tyrosine kinase activity may stimulate autophosphorylation and may affect a biological activity mediated by HER4. It has been observed in systems described in Section 12 and 13 that binding of HER4 ligands to HER4 triggers tyrosine phosphorylation and affects differentiation of breast cancer cells.

The HER4 ligands of the present invention include NDF, a 44 kDa glycoprotein isolated from ras-transformed rat fibroblasts (Wen *et al.*, 1992, Cell 69:559-572); heregulin, its human homologue, which exists as multiple isoforms (Peles *et al.*, 1992, Cell 69:205-218 and Holmes *et al.*, 1992, Science 256:1205-1210) including p45, a 45K heparin-binding glycoprotein that shares several features with the heregulin-family of proteins including molecular weight, ability to induce differentiation of breast cancer cells, activation of tyrosine phosphorylation in MDA-MB453 cells, and N-terminal amino acid sequence (Section 13, *infra*), gp30, and p75 (Lupu *et al.*, 1990,

Science 249:1552-1555 and Lupu *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:2287-2291).

HER4 ligands of the present invention can be prepared by synthetic or recombinant means, or can be isolated from natural sources. The HER4 ligand of the present invention may contain deletions, additions or substitutions of amino acid residues relative to the sequence of NDF, p45 or other heregulins or any HER4 ligand known in the art as long as the ligand maintains HER4 receptor binding and tyrosine kinase activation capacity. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the resides involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

5.5.1. Recombinant Expression of HER4 Ligands

The HER4 ligands of the present invention may be produced by the cloning and expression of DNA encoding the desired HER4 ligand. Such DNA may be ligated into a number of expression vectors well known in the art and suitable for use in a number of acceptable host organisms, in fused or mature form, and may contain a signal sequence to permit secretion. Both prokaryotic and eukaryotic host expression systems may be employed in the production of recombinant HER4 ligands. For example, a HER4 ligand precursor coding sequence or its functional equivalent may be used in a host cell

capable of processing the precursor correctly. Alternatively, the coding sequence for a mature HER4 ligand may be used to directly express the mature HER4 ligand molecule. Functional equivalents of the HER4 ligand precursor coding sequence include any DNA sequence which, when expressed inside the appropriate host cell, is capable of directing the synthesis, processing and/or export of the HER4 ligand.

Production of a HER4 ligand using recombinant DNA technology may be divided into a four-step process for the purposes of description: (1) isolation or generation of DNA encoding the desired HER4 ligand; (2) construction of an expression vector capable of directing the synthesis of the desired HER4 ligand; (3) transfection or transformation of appropriate host cells capable of replicating and expressing the HER4 ligand coding sequence and/or processing the initial product to produce the desired HER4 ligand; and (4) identification and purification of the desired HER4 ligand product.

5.5.2. Isolation of HER4 Encoding DNA

HER4 ligand-encoding nucleic acid sequences may be obtained from human hepatocellular carcinoma cell lines, specifically the HepG2 cells available from the ATCC, accession number HB 8065. In addition, a number of human cell sources are suitable for obtaining HER4 ligand nucleic acids, including MDA-MB-231 cells available from the ATCC, accession number HTB 26, brain tissue (Falls *et al.*, 1993, Cell 72:801-815 and Marchionni *et al.*, 1993 Nature 362:312-318), and any cell source capable of producing an activity capable of binding to the 180K transmembrane protein, HER4/p180^{erbB4}, encoded by the HER4/ERBB4 gene and activating tyrosine kinase activity.

Methods useful in assaying for the identification of HER4 ligands is disclosed in Section 5.8., *infra*.

The techniques disclosed in Sections 5.3.2. and 5.3.3., *infra* apply to the construction of HER4 ligand expression vectors and identification of recombinant transformants expressing HER4 ligand gene products.

5.5.3. Anti-HER4 Ligand Antibodies

The present invention is also directed to polyclonal and monoclonal antibodies which recognize eptitopes of HER4 ligand polypeptides. Anti-HER4 ligand antibodies are expected to have a variety of useful applications in the field of oncology. Briefly, anti-HER4 ligand antibodies may be used for the detection and quantification of HER4 ligand polypeptide expression in cultured cells, tissue samples, and *in vivo*. For example, monoclonal antibodies recognizing epitopes from different parts of the HER4 ligand structure may be used to detect and/or distinguish binding from non-binding regions of the ligand. Anti-HER4 ligand antibody preparations are also envisioned as useful biomodulatory agents capable of effectively treating particular human cancers. An anti-HER4 ligand antibody could be used to block signal transduction mediated through HER4, thereby inhibiting undesirable biological responses. In addition to the various diagnostic and therapeutic utilities of anti-HER4 ligand antibodies, a number of industrial and research applications will be obvious to those skilled in the art, including, for example, the use of anti-HER4 ligand antibodies as affinity reagents for the purification of HER4 ligand polypeptides, and as immunological probes for elucidating the biosynthesis, metabolism and biological functions of HER4 ligands.

Anti-HER4 ligand antibodies may be useful for influencing cell functions and behaviors which are directly or indirectly mediated by HER4. As an example, modulation of HER4 biological activity with anti-HER4 ligand antibodies may influence HER2 activation and, as a consequence, modulate intracellular signals generated by HER2. In this regard, anti-HER4 ligand antibodies may be useful to effectively block ligand-induced, HER4-mediated activation of HER2, thereby affecting HER2 biological activity. Conversely, anti-HER4 ligand antibodies capable of acting as HER4 ligands may be used to trigger HER4 biological activity and/or initiate a ligand-induced, HER4-mediated effect on HER2 biological activity, resulting in a cellular response such as differentiation, growth inhibition, etc.

Additionally, anti-HER4 ligand antibodies conjugated to cytotoxic compounds may be used to selectively target such compounds to tumor cells expressing HER4, resulting in tumor cell death and reduction or eradication of the tumor.

Various procedures known in the art may be used for the production of antibodies to epitopes of HER4 ligand (see Section 5.4, *supra*).

5.6. Diagnostic Methods

The invention also relates to the detection of human neoplastic conditions, particularly carcinomas of epithelial origin, and more particularly human breast carcinomas. In one embodiment, oligomers corresponding to portions of the consensus HER4 cDNA sequence provided in FIG. 1A and 1B are used for the quantitative detection of HER4 mRNA levels in a human biological sample, such as blood, serum, or tissue biopsy samples, using a suitable hybridization or PCR

format assay, in order to detect cells or tissues expressing abnormally high levels of HER4 as an indication of neoplasia. In a related embodiment, detection of HER4 mRNA may be combined with the detection HER2 mRNA overexpression, using appropriate HER2 sequences, to identify neoplasias in which a functional relationship between HER2 and HER4 may exist.

In another embodiment, labeled anti-HER4 antibodies or antibody derivatives are used to detect the presence of HER4 in biological samples, using a variety of immunoassay formats well known in the art, and may be used for in situ diagnostic radioimmunoimaging. Current diagnostic and staging techniques do not routinely provide a comprehensive scan of the body for metastatic tumors. Accordingly, anti-HER4 antibodies labeled with, for example, fluorescent, chemiluminescent, and radioactive molecules may overcome this limitation. In a preferred embodiment, a gamma-emitting diagnostic radionuclide is attached to a monoclonal antibody which is specific for an epitope of HER4, but not significantly cross-reactive with other EGFR-family members. The labeled antibody is then injected into a patient systemically, and total body imaging for the distribution and density of HER4 molecules is performed using gamma cameras, followed by localized imaging using computerized tomography or magnetic resonance imaging to confirm and/or evaluate the condition, if necessary. Preferred diagnostic radionuclides include but are not limited to technetium-99m, indium-111, iodine-123, and iodine-131.

Recombinant antibody-metallothionein chimeras (Ab-MTs) may be generated as recently described (Das

et *al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:9749-53). Such Ab-MTs can be loaded with technitium-99m by virtue of the metallothionein chelating function, and may offer advantages over chemically conjugated chelators. In particular, the highly conserved metallothionein structure may result in minimal immunogenicity.

5.7. Assays for the Identification of HER4 Ligands

Cell lines overexpressing a single member of the EGFR-family can be generated by transfection of a variety of parental cell types with an appropriate expression vector as described in Section 7., *infra*. Candidate ligands, or partially purified preparations, may be applied to such cells and assayed for receptor binding and/or activation. For example, a CHO-KI cell line transfected with a HER4 expression plasmid and lacking detectable EGFR, HER2, or HER3 may be used to screen for HER4-specific ligands. A particular embodiment of such a cell line is described in Section 7., *infra*, and has been deposited with the ATCC (CHO/HER4 21-2). Ligands may be identified by detection of HER4 autophosphorylation, stimulation of DNA synthesis, induction of morphologic differentiation, relief from serum or growth factor requirements in the culture media, and direct binding of labeled purified growth factor. The invention also relates to a bioassay for testing potential analogs of HER4 ligands based on a capacity to affect a biological activity mediated by the HER4 receptor.

5.8. Use Of The Invention in Cancer Therapy

5.8.1. Targeted Cancer Therapy

The invention is also directed to methods for the treatment of human cancers involving abnormal expression and/or function of HER4 and cancers in which HER2 overexpression is combined with the proximate expression of HER4, including but not limited to human breast carcinomas and other neoplasms overexpressing HER4 or overexpressing HER2 in combination with expression of HER4. The cancer therapy methods of the invention are generally based on treatments with unconjugated, toxin- or radionuclide- conjugated HER4 antibodies, ligands, and derivatives or fragments thereof. In one specific embodiment, such HER4 antibodies or ligands may be used for systemic and targeted therapy of certain cancers overexpressing HER2 and/or HER4, such as metastatic breast cancer, with minimal toxicity to normal tissues and organs. Importantly, in this connection, an anti-HER2 monoclonal antibody has been shown to inhibit the growth of human tumor cells overexpressing HER2 (Bacus *et al.*, 1992, Cancer Res. 52:2580-89). In addition to conjugated antibody therapy, modulation of heregulin signaling through HER4 provides a means to affect the growth and differentiation of cells overexpressing HER2, such as certain breast cancer cells, using HER4-neutralizing monoclonal antibodies, NDF/HER4 antagonists, monoclonal antibodies or ligands which act as super-agonists for HER4 activation, or agents which block the interaction between HER2 and HER4, either by disrupting heterodimer formation or by blocking HER-mediated phosphorylation of the HER2 substrate.

For targeted immunotoxin-mediated cancer therapy, various drugs or toxins may be conjugated to anti-HER4 antibodies and fragments thereof, such as plant and bacterial toxins. For example, ricin, a cytotoxin from the Ricinis communis plant may be conjugated to an anti-HER4 antibody using methods known in the art (*e.g.*, Blakey *et al.*, 1988, Prog. Allergy 45:50-90; Marsh and Neville, 1988, J. Immunol. 140:3674-78). Once ricin is inside the cell cytoplasm, its A chain inhibits protein synthesis by inactivating the 60S ribosomal subunit (May *et al.*, 1989, EMBO J. 8:301-08). Immunotoxins of ricin are therefore extremely cytotoxic. However, ricin immunotoxins are not ideally specific because the B chain can bind to virtually all cell surface receptors, and immunotoxins made with ricin A chain alone have increased specificity. Recombinant or deglycosylated forms of the ricin A chain may result in improved survival (*i.e.*, slower clearance from circulation) of the immunotoxins. Methods for conjugating ricin A chain to antibodies are known (*e.g.*, Vitella and Thorpe, in: Seminars in Cell Biology, pp 47-58; Saunders, Philadelphia 1991). Additional toxins which may be used in the formulation of immunotoxins include but are not limited to daunorubicin, methotrexate, ribosome inhibitors (*e.g.*, trichosanthin, trichokirin, gelonin, saporin, mormordin, and pokeweed antiviral protein) and various bacterial toxins (*e.g.*, *Pseudomonas exotoxin*). Immunotoxins for targeted cancer therapy may be administered by any route which will result in antibody interaction with the target cancer cells, including systemic administration and injection directly to the site of tumor. Another therapeutic strategy may be the administration of immunotoxins by sustained-release systems, such as

semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release immunotoxic molecules for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

For targeted radiotherapy using anti-HER4 antibodies, preferred radionuclides for labeling include alpha, beta, and Auger electron emitters. Examples of alpha emitters include astatine 211 and bismuth 212; beta emitters include iodine 131, rhenium 188, copper 67 and yttrium 90; and iodine 125 is an example of an Auger electron emitter.

Similarly as suggested for the use of toxin-conjugated antibodies as therapeutic agents for targeted cancer therapy, purified ligand molecules may be chemically conjugated to cytotoxic substances. In addition, recombinant chimeric polypeptides comprising a HER4 binding (=ligand) portion fused to all or part of a cytotoxin may be engineered by constructing vectors comprising DNA encoding the ligand in reading frame with DNA encoding the toxin or part thereof. Such recombinant ligand-toxins may be used to specifically target HER4 expressing cancer cells. A particular embodiment of such a ligand-toxin is disclosed herein and described in more detail in Sections 5.8.2., *infra*, and Section 15, *infra*.

5.8.2. The Generation Of A Heregulin-toxin Specifically Targeting HER4 Expressing Tumor Cells

Another aspect of the invention relates to the development of a strategy to selectively target and kill HER4 expressing tumor cells. More particularly, HER4 expressing tumor cells may be specifically targeted and killed by contacting such tumor cells with a fusion protein comprising a cytotoxic polypeptide covalently linked to a polypeptide which is capable of activating HER4 expressed on such cells.

In a specific embodiment described by way of example in Section 15, *infra*, a fusion protein comprising a chimeric heregulin β2 ligand and the cytotoxic substance PE40 is generated by expression of the corresponding chimeric coding sequence. PE40 is a derivative of the *Pseudomonas* exotoxin PE, a potent cell killing agent made by *Pseudomonas aeruginosa* (Fitzgerald *et al.*, 1980, Cell 21:867-873). The wildtype protein PE contains three domains whose functions are cell recognition, membrane translocation, and ADP ribosylation of elongation factor 2. It kills cells by binding to a cell surface receptor, entering the cell via an endocytotic vesicle and catalyzing ADP-ribosylation of elongation factor 2. The derivative PE40 lacks the cell binding function of the wildtype protein, but still exhibits strong cytotoxic activity. Generation of PE40 fusion proteins with specific cell targeting molecules have been described (Kondo *et al.*, 1988, J. Biol. Chem. 263:9470-9475 (PE40 fusions with different monoclonal antibodies); Friedman *et al.*, 1993, Cancer Res. 53:334-339 (BR96/PE40 fusions); U.S. Pat. No. 5206353 (CD4/PE40 fusions); U.S. Pat. No. 5082927 (IL-4/PE40 fusions) and U.S. Pat. No. 4892827 (TGF-α/PE40 and IL-2/PE40 fusions)).

The chimeric heregulin-toxin protein HAR-TX β2 described in Section 15, *infra*, contains the amphiregulin (AR) leader sequence thereby facilitating the purification of the recombinant protein. As confirmed by applicants' data, the AR leader has no influence on the binding specificity of the recombinant heregulin-toxin. Related embodiments include, for example, PE40 linked to other members of the heregulin family, like heregulin-β1 and heregulin-α, and other molecules capable of activating HER4.

In a cytotoxicity assay with cultured tumor cell lines, the applicants demonstrate specificity of the cytotoxic effect of the chimeric heregulin-PE40 protein to HER4 expressing cancer cells; they include but are not limited to prostate carcinoma, bladder carcinoma, and a considerable number of different breast cancer types, including breast carcinoma cells with amplified HER2 expression. The bifunctional retention of both the specificity of the cell binding portion of the molecule and the cytotoxic potential of PE40 provides a very potent and targeted reagent.

An effective therapeutic amount of heregulin-toxin will depend upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, dosages should be titrated and the route of administration modified as required to obtain the optimal therapeutic effect. A typical daily dosage may be in the range of 0.1 mg/kg - 1 mg/kg, preferably between 0.1 and 0.5 mg/kg, with intravenous administration. For regression of solid tumors, it may take 3-5 doses, with schedules such as 3 doses, each four days apart. Also the use of sustained-release preparations (see Section 5.8.1., *supra*) may be considered for administration of the reagent. The therapeutic efficacy of heregulin-toxin

may be between 2 and 10, which means that a tumor regression effect would be expected between 2- and 10-fold below the toxic dose (see Section 15, *infra*). Desirably, the heregulin-toxin will be administered at a dose and frequency that achieves the desired therapeutic effect, which can be monitored using conventional assays.

Cancer therapy with heregulin-toxins of the invention may be combined with chemotherapy, surgery, and radiation therapy, depending on the type of tumor. One advantage of using a low molecular weight toxin drug is that they are capable of targeting metastatic lesions that cannot be located and removed by surgery. Heregulin-toxins may also be particularly useful on patients that are MDR (Multi Drug Resistance) positive since their mechanism of action is not inhibited by the p-glycoprotein pump of MDR positive cells as are many standard cancer therapeutic drugs.

5.9. Other Therapeutic Use Of HER4 Ligands

Additional therapeutic uses of HER4 ligands may include other diseases caused by deficient HER4 receptor tyrosine kinase activation rather than by hyperactivation. In this regard, type II diabetes mellitus is the consequence of deficient insulin-mediated signal transduction, caused by mutations in the insulin-receptor, including mutations in the ligand-binding domain (Taira *et al.*, 1889, Science 245:63-66; Odawara *et al.*, 1989, Science 245:66-68; Obermeier-Kusser *et al.*, 1989, J. Biol. Chem. 264:9497-9504). Such diseases might be treated by administration of modified ligands or ligand-analogues which re-establish a functional ligand-receptor interaction.

5.10. HER4 Analogues

The production and use of derivatives, analogues and peptides related to HER4 are also envisioned and are within the scope of the invention. Such derivatives, analogues and peptides may be used to compete with native HER4 for binding of HER4 specific ligand, thereby inhibiting HER4 signal transduction and function. The inhibition of HER4 function may be utilized in several applications, including but not limited to the treatment of cancers in which HER4 biological activity is involved.

In a specific embodiment, a series of deletion mutants in the HER4 nucleotide coding sequence depicted in FIG. 1A and 1B may be constructed and analyzed to determine the minimum amino acid sequence requirements for binding of a HER4 ligand. Deletion mutants of the HER4 coding sequence may be constructed using methods known in the art which include but are not limited to use of nucleases and/or restriction enzymes; site-directed mutagenesis techniques, PCR, etc. The mutated polypeptides expressed may be assayed for their ability to bind HER4 ligand.

The DNA sequence encoding the desired HER4 analogue may then be cloned into an appropriate expression vector for overexpression in either bacteria or eukaryotic cells. Peptides may be purified from cell extracts in a number of ways including but not limited to ion-exchange chromatography or affinity chromatography using HER4 ligand or antibody. Alternatively, polypeptides may be synthesized by solid phase techniques followed by cleavage from resin and purification by high performance liquid chromatography.

6. Example: Isolation of cDNAs Encoding HER4

EGFR and the related proteins, HER2, HER3, and Xmrk exhibit extensive amino acid homology in their tyrosine kinase domains (Kaplan *et al.*, 1991, Nature 350:158-160; Wen *et al.*, 1992, Cell 69:559-72; Holmes *et al.*, 1992, Science 256:1205-10; Hirai *et al.*, Science 1987 238:1717-20). In addition, there is strict conservation of the exon-intron boundaries within the genomic regions that encode these catalytic domains (Wen *et al.*, *supra*; Lindberg and Hunter, 1990, Mol. Cell. Biol. 10:6316-24; and unpublished observations). Degenerate oligonucleotide primers were designed based on conserved amino acids encoded by a single exon or adjacent exons from the kinase domains of these four proteins. These primers were used in a polymerase chain reaction (PCR) to isolate genomic fragments corresponding to murine EGFR, *erb*B2 and *erb*B3. In addition, a highly related DNA fragment (designated MER4) was identified as distinct from these other genes. A similar strategy was used to obtain a cDNA clone corresponding to the human homologue of MER4 from the breast cancer cell line, MDA-MB-453. Using this fragment as a probe, several breast cancer cell lines and human heart were found to be an abundant source of the EGFR-related transcript. cDNA libraries were constructed using RNA from human heart and MDA-MB-453 cells, and overlapping clones were isolated spanning the complete open reading frame of HER4/*erb*B4.

6.1. Materials and Methods

6.1.1. Molecular Cloning

Several pools of degenerate oligonucleotides were synthesized based on conserved sequences from EGFR-family members (Table I) (5'-ACNGTNTGGGARYTNAYHAC-3'

[SEQ ID No:14]; 5'-CAYGTNAARATHACNGAYTTYGG-3' [SEQ ID No:16]; 5'-GACGAATTCCNATHAARTGGATGGC-3' [SEQ ID No:17]; 5'-AANGTCATNARYTCCCA-3' [SEQ ID No:18]; 5'-TCCAGNGCGATCCAYTTDATNGG-3' [SEQ ID No:19]; 5'-GGRTCDATCATCCARCCT-3' [SEQ ID No:20]; 5'-CTGCTGTCAGCATCGATCAT-3' [SEQ ID No:21]; TVWELMT [SEQ ID No:22]; HVKITDFG [SEQ ID No:23]; PIKWMA [SEQ ID No:13]; VYMIILK [SEQ ID No:24]; WELMTF [SEQ ID No:25]; PIKWMALE [SEQ ID No:26]; CWMIDP [SEQ ID No:27]. Total genomic DNA was isolated from subconfluent murine K1735 melanoma cells and used as a template with these oligonucleotide primers in a 40 cycle PCR amplification. PCR products were resolved on agarose gels and hybridized to 32P-labeled probes from the kinase domain of human EGFR and HER2. Distinct DNA bands were isolated and subcloned for sequence analysis. Using the degenerate oligonucleotides H4VWELM and H4VYMIIL as primers in a PCR amplification (Plowman *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:4905-09), one clone (MER4-85) was identified that contained a 144 nucleotide insert corresponding to murine *erb*B4. This 32P-labeled insert was used to isolate a 17-kilobase fragment from a murine T-cell genomic library (Stratagene, La Jolla, CA) that was found to contain two exons of the murine *erb*B4 gene. A specific oligonucleotide (4M3070) was synthesized based on the DNA sequence of an *erb*B4 exon, and used in a PCR protocol with a degenerate 5'-oligonucleotide (H4PIKWMA) on a template of single stranded MDA-MB-453 cDNA. This reaction generated a 260 nucleotide fragment (pMDAPIK) corresponding to human HER4. cDNA libraries were constructed in lambda ZAP II (Stratagene) from oligo(dT)- and specific-primed MDA-MB453 and human heart RNA (Plowman *et al.*, *supra*; Plowman *et al.*, 1990, Mol. Cell. Biol. 10:1969-81).

HER4-specific clones were isolated by probing the libraries with the ^{32}P-labeled insert from pMDAPIK. To complete the cloning of the 5'-portion of HER4, we used a PCR strategy to allow for rapid amplification of cDNA ends (Plowman *et al.*, *supra*; Frohman *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. 85:8998-9002). All cDNA clones and several PCR generated clones were sequenced on both strands using T7 polymerase with oligonucleotide primers (Tabor and Richardson, 1987, Proc. Natl. Acad. Sci. U.S.A. 84:4767-71).

TABLE I

OLIGONUCLEOTIDE PREPARATIONS FOR CLONING HER4

Designation	Nucleotide Sequence[1]	Degeneracy	Encoded Sequence	Orientation	Seq.ID No.
H4TVWELM	5'-ACNGTNTGGGARYTNAYHAC-3'	256-fold	TVWELMT	sense	14
H4KITDFG	5'-CAYGTNAARATHACNGAYTTYGG-3'	768-fold	HVKITDFG	sense	15
H4PIKWMA	5'-GACGAATTCCNATHAARTGGATGGC	48-fold	PIKWMA	sense	16
H4VYMIIL	5'-ACAYTTNARDATDATCATRTANAC-3'	576-fold	VYMIILK	antisense	17
H4WELMTF	5'-AANGTCATNARYTCCCA-3'	32-fold	WELMTF	antisense	18
H4PIKWMA	5'-TCCAGNGCGATCCAYTTDATNGG-3'	96-fold	PIKWMALE	antisense	19
H4CWMIDP	5'-GGRTCDATCATCCARCCT-3'	12-fold	CWMIDP	antisense	20
4M3070	5'-CTGCTGTCAGCATCGATCAT-3'	zero	*erbB4* exon	antisense	21

[1]Degenerate nucleotide residue designations:
D = A, G, or T;
H = A, C, or T;
N = A, C, G, or T;
R = A or G; and
Y = C or T.

6.1.2. Northern Blot Analysis

3'- and 5'-HER4 specific [α^{32}P]UTP-labeled antisense RNA probes were synthesized from the linearized plasmids pHt1B1.6 (containing an 800 bp HER4 fragment beginning at nucleotide 3098) and p5'H4E7 (containing a 1 kb fragment from the 5'-end of the HER4 sequence), respectively. For tissue distribution analysis (Section 6.2.3., *infra*), the Northern blot (Clontech, Palo Alto, CA) contained 2 Mg

poly(A)+ mRNA per lane from 8 human tissue samples immobilized on a nylon membrane. The filter was prehybridized at 60° C for several hours in RNA hybridization mixture (50% formamide, 5x SSC, 0.5% SDS, 10x Denhardt's solution, 100 μg/ml denatured herring sperm DNA, 100 μg/ml tRNA, and 10 μg/ml polyadenosine) and hybridized in the same buffer at 60° C, overnight with 1-1.5 x 106 cpm/ml of 32P-labeled antisense RNA probe. The filters were washed in 0.1XSSC/0.1% SDS, 65° C, and exposed overnight on a PhosphoImager (Molecular Dynamics, Sunnyvale, CA).

6.1.3. Semi-Quantitative PCR Detection of HER4

RNA was isolated from a variety of human cell lines, fresh frozen tissues, and primary tumors. Single stranded cDNA was synthesized from 10 μg of each RNA by priming with an oligonucleotide containing a T17 track on its 3'-end

(XSCT17:5'GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3') [SEQ ID No:28].

1% or 5% of each single strand template preparation was then used in a 35 cycle PCR reaction with two HER4-specific oligonucleotides:

4H2674: 5'-GAAGAAAGACGACTCGTTCATCGG-3' [SEQ ID No:29],

and

4H2965: 5'-GACCATGACCATGTAAACGTCAATA-3' [SEQ ID No:30].

Reaction products were electrophoresed on 2% agarose gels, stained with ethidium bromide and photographed on a UV light box. The relative intensity of the 291-bp HER4-specific bands were estimated for each sample as shown in Table II.

6.2. Results

6.2.1. Sequence Analysis of cDNA Clones Encoding HER4

cDNA clones encoding parts of the HER4 coding and non-coding nucleotide sequences were isolated by PCR cloning according to the method outlined in Section 6.1.1., *supra*. The complete HER4 nucleotide sequence assembled from these cDNAs is shown in FIG. 1A and 1B and contains a single open reading frame encoding a polypeptide of 1308 amino acids. The HER4 coding region is flanked by a 33 nucleotide 5'-untranslated region and a 1517 nucleotide 3'-untranslated region ending with a poly(A) tail. A 25 amino acid hydrophobic signal sequence follows a consensus initiating methionine at position number 1 in the amino acid sequence depicted in FIG. 1A and 1B. In relation to this signal sequence, the mature HER4 polypeptide would be predicted to begin at amino acid residue number 26 in the sequence depicted in FIG. 1A and 1B (Gln), followed by the next 1283 amino acids in the sequence. Thus the prototype mature HER4 of the invention is a polypeptide of 1284 amino acids, having a calculated Mr of 144,260 daltons and an amino acid sequence corresponding to residues 26 through 1309 in FIG. 1A and 1B.

Comparison of the HER4 nucleotide and deduced amino acid sequences (FIG. 1A and 1B) with the available DNA and protein sequence databases indicated that the HER4 nucleotide sequence is unique, and revealed a 60/64 amino acid identity with HER2 and a 54/54 amino acid identity to a fragment of a rat EGFR homolog, tyro-2.

6.2.2. Sequence Analysis of Related cDNAs

Several cDNAs encoding polypeptides related to the prototype HER4 polypeptide (FIG. 1A and 1B) were

also isolated from the MDA-MB-453 cDNA library and comprised two forms.

The first alternative type of cDNA was identical to the consensus HER4 nucleotide sequence up to nucleotide 3168 (encoding Arg at amino acid position 1045 in the FIG. 1A and 1B) and then abruptly diverges into an apparently unrelated sequence (FIG. 2A and 2B, FIG. 4). Downstream from this residue the open reading frame continues for another 13 amino acids before reaching a stop codon followed by a 2 kb 3′-untranslated sequence and poly(A) tail. This cDNA would be predicted to result in a HER4 variant having the C-terminal autophosphorylation domain of the prototype HER4 deleted.

A second type of cDNA was isolated as 4 independent clones each with a 3′-sequence identical to the HER4 consensus, but then diverging on the 5′-side of nucleotide 2335 (encoding Glu at amino acid position 768 in the FIG. 1A and 1B), continuing upstream for only another 114-154 nucleotides (FIG. 3, FIG. 5). Nucleotide 2335 is the precise location of an intron-exon junction in the HER2 gene (Coussens *et al.*, 1985, Science 230:1132-39; Semba *et al.*, 1985, Proc. Natl. Acad. Sci. U.S.A. 82:6497-6501), suggesting these cDNAs could be derived from mRNAs that have initiated from a cryptic promoter within the flanking intron. These 5′-truncated transcripts contain an open reading frame identical to that of the HER4 cDNA sequence of FIG. 1A and 1B, beginning with the codon for Met at amino acid position 772 in FIG. 1A and 1B. These cDNAs would be predicted to encode a cytoplasmic HER4 variant polypeptide that initiates just downstream from the ATP-binding domain of the HER4 kinase.

6.2.3. Human Tissue Distribution of HER4 Expression

Northern blots of poly(A)+ mRNA from human tissue samples were hybridized with antisense RNA probes to the 3'-end of HER4, encoding the autophosphorylation domain, as described in Section 6.1.2., *supra*. A HER4 mRNA transcript of approximately 6kb was identified, and was found to be most abundant in the heart and skeletal muscle (FIG. 8, Panel 1). An mRNA of greater than approximately 15 kb was detected in the brain, with lower levels also detected in heart, skeletal muscle, kidney, and pancreas tissue samples.

The same blot was stripped and rehybridized with a probe from the 5'-end of HER4, within the extracellular domain coding region, using identical procedures. This hybridization confirmed the distribution of the 15 kb HER4 mRNA species, and detected a 6.5 kb mRNA species in heart, skeletal muscle, kidney, and pancreas tissue samples (FIG. 8, Panel 2) with weaker signals in lung, liver, and placenta. In addition, minor transcripts of 1.7-2.6 kb were also detected in pancreas, lung, brain, and skeletal muscle tissue samples. The significance of the different sized RNA transcripts is not known.

Various human tissues were also examined for the presence of HER4 mRNA using the semi-quantitative PCR assay described in Section 6.1.3., *supra*. The results are shown in Table II, together with results of the assay on primary tumor samples and neoplastic cell lines (Section 6.2.4., immediately below). These results correlate well with the Northern and solution hybridization analysis results on the selected RNA samples. The highest levels of HER4 transcript expression were found in heart, kidney, and brain tissue samples. In addition, high levels of HER4 mRNA expression were found in parathyroid, cerebellum,

pituitary, spleen, testis, and breast tissue samples. Lower expression levels were found in thymus, lung, salivary gland, and pancreas tissue samples, Finally, low or negative expression was observed in liver, prostate, ovary, adrenal, colon, duodenum, epidermis, and bone marrow samples.

6.2.4. HER4 mRNA Expression in Primary Tumors and Various Cell Lines of Neoplastic Origin

HER4 mRNA expression profiles in several primary tumors and a number of cell lines of diverse neoplastic origin were determined with the semi-quantitative PCR assay (Section 6.1.3, *supra*) using primers from sequences in the HER4 kinase domain. The results are included in Table II. This analysis detected the highest expression of HER4 RNA in 4 human mammary adenocarcinoma cell lines (T-47D, MDA-MB-453, BT-474, and H3396), and in neuroblastoma (SK-N-MC), and pancreatic carcinoma (Hs766T) cell lines. Intermediate expression was detected in 3 additional mammary carcinoma cell lines (MCF-7, MDA-MB-330, MDA-MB-361). Low or undetectable expression was found in other cell lines derived from carcinomas of the breast (MDB-MB-231, MDA-MB-157, MDA-MB-468, SK-BR-3), kidney (Caki-1, Caki-2, G-401), liver (SK-HEP-1, HepG2), pancreas (PANC-1, AsPC-1, Capan-1), colon (HT-29), cervix (CaSki), vulva (A-41), ovary (PA-1, Caov-3), melanoma (SK-MEL-28), or in a variety of leukemic cell lines. Finally, high level expression was observed in Wilms (kidney) and breast carcinoma primary tumor samples.

TABLE II

HER4 EXPRESSION BY PRC ANALYSIS

VERY STRONG	STRONG	MEDIUM
T47D (breast)	MDA-MB-453 (breast)	MCF-7 (breast)
	BT-474 (breast)	MDA-MB-330 (breast)
	H3396 (breast)	MDA-MB-157 (breast)
	Hs766T (pancreatic)	JEG-3
(choriocarcinoma)		
	SK-N-MC (neural)	HEPM (palate)
	Wilms Tumor (kidney)	458(medullablastoma)
		Breast Carcinoma
Kidney	Brain	Skeletal Muscle
Heart	Cerebellum	Thymus
Parathyroid	Pituitary	Pancreas
	Breast	Lung
	Testis	Salivary Gland
	Spleen	

WEAK	NEGATIVE
MDB-MB-231 (breast)	MDA-MB-468 (breast)
MDA-MB-157 (breast)	G-401 (kidney)
SK-BR-3 (breast)	HepG2 (liver)
A-431 (vulva)	PANC-1 (pancreas)
Caki-1 (kidney)	AsPC-1(pancreas)
Caki-2 (kidney)	Capan-1 (pancreas)
SK-HEP-1 (liver)	HT-29 (colon)
THP-1 (macrophage)	CaSki (cervix)
	PA-1 (ovary)
Prostate	Caov-3 (ovary)
Adrenal	SK-MEL-28 (melanoma)
Ovary	HUF (fibroblast)
Colon	H2981 (lung)
Placenta	Ovarian tumor
	GEO (colon)
	ALL bone marrow
	AML bone marrow
	Duodenum
	Epidermis
	Liver
	Bone marrow stroma

7. Example: Recombinant Expression of HER4

7.1. Materials and Methods

7.1.1. CHO-KI Cells and Culture Conditions

CHO-KI cells were obtained from the ATCC (Accession Number CCL 61). These cells lack any detectable EGFR, HER2, or HER3 by immunoblot, tyrosine

phosphorylation, and ^{35}S-labeled immunoprecipitation analysis. Transfected cell colonies expressing HER4 were selected in glutamine-free Glasgow modified Eagle's medium (GMEM-S, Gibco) supplemented with 10% dialyzed fetal bovine serum an increasing concentrations of methionine sulfoximine (Bebbington, 1991, in Methods: A Companion to Methods in Enzymology 2:136-145 Academic Press).

7.1.2. Expression Vector Construction and Transfections

The complete 4 kilobase coding sequence of prototype HER4 was reconstructed and inserted into a glutamine synthetase expression vector, pEE14, under the control of the cytomegalovirus immediate-early promoter (Bebbington, *supra*) to generate the HER4 expression vector pEEHER4. This construct (pEEHER4) was linearized with MluI and transfected into CHO-KI cells by calcium phosphate precipitation using standard techniques. Cells were placed on selective media consisting of GMEM-S supplemented with 10% dialyzed fetal bovine serum and methionine sulfoximine at an initial concentration of 25 μM (L-MSX) as described in Bebbington, *supra*, for the selection of initial resistant colonies. After 2 weeks, isolated colonies were transferred to 48-well plates and expanded for HER4 expression immunoassays as described immediately below. Subsequent rounds of selection using higher concentrations of MSX were used to isolate cell colonies tolerating the highest concentrations of MSX. A number of CHO/HER4 clones selected at various concentrations of MSX were isolated in this manner.

7.1.3. HER4 Expression Immunoassay

Confluent cell monolayers were scraped into hypotonic lysis buffer (10 mM Tris pH7.4, 1 mM KCl, 2 mM MgCl2) at 4° C, dounce homogenized with 30 strokes, and the cell debris was removed by centrifugation at 3500 x g, 5 min. Membrane fractions were collected by centrifugation at 100,000 x g, 20 min, and the pellet was resuspended in hot Laemmli sample buffer with 2-mercaptoethanol. Expression of the HER4 polypeptide was detected by immunoblot analysis on solubilized cells or membrane preparations using HER2 immunoreagents generated to either a 19 amino acid region of the HER2 kinase domain, which coincidentally is identical to the HER4 sequence (residues 927-945), or to the C-terminal 14 residues of HER2, which share a stretch of 7 consecutive residues with a region near the C-terminus of HER4. On further amplification, HER4 was detected from solubilized cell extracts by immunoblot analysis with PY20 anti-phosphotyrosine antibody (ICN Biochemicals), presumably reflecting autoactivation and autophosphorylation of HER4 due to receptor aggregation resulting from abberantly high receptor density. More specifically, expression was detected by immunobloting with a primary murine monoclonal antibody to HER2 (Neu-Ab3, Oncogene Science) diluted 1:50 in blotto (2.5% dry milk, 0.2% NP40 in PBS) using ^{125}I-goat anti-mouse Ig F(ab')2 (Amersham, UK) diluted 1:500 in blotto as a second antibody. Alternatively, a sheep polyclonal antipeptide antibody against HER2 residues 929-947 (Cambridge Research Biochemicals, Valleystream, NY) was used as a primary immunoreagent diluted 1:100 in blotto with ^{125}I-Protein G (Amersham) diluted 1:200 in blotto as a second antibody. Filters were washed with

blotto and exposed overnight on a phosphoImager (Molecular Dynamics).

7.2. Results

CHO-KI cells transfected with a vector encoding the complete human prototype HER4 polypeptide were selected for amplified expression in media containing increasing concentrations of methionine sulfoximine as outlined in Section 7.1., et seq., *supra*. Expression of HER4 was evaluated using the immunoassay described in Section 7.1.3., *supra*. Several transfected CHO-KI cell clones stably expressing HER4 were isolated. One particular clone, CHO/HER4 21-2, was selected in media supplemented with 250 μM MSX, and expresses high levels of HER4. CHO/HER4 21-2 cells have been deposited with the ATCC.

Recombinant HER4 expressed in CHO/HER4 cells migrated with an apparent Mr of 180,000, slightly less than HER2, whereas the parental CHO cells showed no cross-reactive bands (FIG. 9). In addition, a 130 kDa band was also detected in the CHO/HER4 cells, and presumably represents a degradation product of the 180 kDa mature protein. CHO/HER4 cells were used to identify ligand specific binding and autophosphorylation of the HER4 tyrosine kinase (see Section 9., et seq., *infra*).

8. Example: Assay for Detecting EGFR-Family Ligands

8.1. Cell Lines

A panel of four recombinant cell lines, each expressing a single member of the human EGFR-family, were generated for use in the tyrosine kinase stimulatory assay described in Section 8.2., below. The cell line CHO/HER4 3 was generated as described in Section 7.1.2, *supra*.

CHO/HER2 cells (clone 1-2500) were selected to express high levels of recombinant human $p185^{erbB2}$ by dihydrofolate reductase-induced gene amplification in dhfr-deficient CHO cells. The HER2 expression plasmid, cDNeu, was generated by insertion of a full length HER2 coding sequence into a modified pCDM8 (Invitrogen, San Diego, CA) expression vector (Seed and Aruffo, 1987, Proc. Natl. Adad. Sci. U.S.A. 84:3365-69) in which an expression cassette from pSV2DHFR (containing the murine dhfr cDNA driven by the SV40 early promoter) has been inserted at the pCDM8 vector's unique BamHI site. This construct drives HER2 expression from the CMV immediate-early promoter.

NRHER5 cells (Velu *et al.*, 1987, Science 1408-10) were obtained from Dr. Hsing-Jien Kung (Case Western Reserve University, Cleveland, OH). This murine cell line was clonally isolated from NR6 cells infected with a retrovirus stock carrying the human EGFR, and was found to have approximately 10^6 human EGFRs per cell.

The cell line 293/HER3 was selected for high level expression of $p160^{erbB3}$. The parental cell line, 293 human embryonic kidney cells, constitutively expresses adenovirus E1a and have low levels of EGFR expression. This line was established by cotransfection of linearized cHER3 (Plowman *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:4905-09) and pMC1neoPolyA (neomycin selectable marker with an Herpes simplex thymidine kinase promoter, Stratagene), with selection in DMEM/F12 media containing 500μg/ml G418.

8.2. Tyrosine Kinase Stimulation Assay

Cells were plated in 6-well tissue culture plates (Falcon), and allowed to attach at 37° C for 18-24 hr. Prior to the assay, the cells were changed to serum-free media for at least 1 hour. Cell monolayers were then incubated with the amounts of ligand preparations indicated in Section 7.3., below for 5 min at 37° C. Cells were then washed with PBS and solubilized on ice with 0.5 ml PBSTDS containing phosphatase inhibitors (10 mM NaHPO4, 7.25, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.2% sodium azide, 1 mM NaF, 1 mM EGTA, 4 mM sodium orthovanadate, 1% aprotinin, 5 mg/ml leupeptin). Cell debris was removed by centrifugation (12000 x g, 15 min, 4° C) and the cleared supernatant reacted with 1 mg murine monoclonal antibody to phosphotyrosine (PY20, ICN Biochemicals, Cleveland, Ohio) for CHO/HER4 and 293/HER3 cells, or 1 mg murine monoclonal antibody to HER2 (Neu-Ab3, Oncogene Sciences) for CHO/HER2 cells, or 1 mg murine monoclonal antibody EGFR-1 to human EGFR (Amersham) for NRHER5 cells. Following a 1 hr incubation at 4° C, 30 μl of a 1:1 slurry (in PBSTDS) of anti-mouse IgG-agarose (for PY20 and Neu-Ab3 antibodies) or protein A-sepharose (for EGFR-R1 antibody) was added and the incubation was allowed to continue an additional 30 minutes. The beads were washed 3 times in PBSTDS and the complexes resolved by electrophoresis on reducing 7% SDS-polyacrylamide gels. The gels were transferred to nitrocellulose and blocked in TNET (10 mM Tris pH7.4, 75 mM NaCl, 0.1% Tween-20, 1 mM EDTA). PY20 antiphosphotyrosine antibody diluted 1:1000 in TNET was used as the primary antibody followed by ^{125}I-goat anti-mouse Ig F(ab')2 diluted 1:500 in TNET. Blots were washed

with TNET and exposed on a phosphorimager (Molecular Dynamics).

8.3. Results

Several EGF-family member polypeptide and ligand preparations were tested for their ability to stimulate tyrosine phosphorylation of each of four EGFR-family receptors expressed in recombinant CHO cells using the tyrosine phosphorylation stimulation assay described in Section 8.2., above. The particular preparations tested for each of the four recombinant cell lines and the results obtained in the assay are tabulated below, and autoradiographs of some of these results are shown in FIG. 10.

TABLE III

STIMULATION OF TYR PHOSPHORYLATION OF EGFR-FAMILY RECEPTORS

PREPARATION	RECOMBINANT CELLS			
	CHO/HER4#3	CHO/HER2	NRHER5	2293/HER3
EGF	-	-	+	-
AMPHIREGULIN	-	-	+	-
TGF-α	-	-	+	-
HB-EGF	-	-	+	-
FRACTION 17*	+	-	-	-
FRACTION 14*	-	-	-	-

* The identification of the HER4 tryrosine kinase stimulatory activity within the conditioned media of HepG2 cells and the isolation of these preparations is described in Section 9, *infra*.

The results indicate that EGF, AR, TGF-α, and HB-EGF, four related ligands which mediate their growth regulatory signals in part through interaction with

EGFR, were able to stimulate tyrosine phosphorylation of EGFR expressed in recombinant NIH3T3 cells (for EGF, see FIG. 10, Panel 3, lane 2), but not HER4, HER2, or HER3 expressed in recombinant CHO or 293 cells (FIG. 10, Panel 1, 2, 4, lanes 2 and 3). Additionally, as discussed in more detail below, the assay identified a HepG2-derived preparation (fraction 17) as a HER4 ligand capable of specifically stimulating tyrosine phoshorylation of HER4 expressed in CHO/HER4 cells alone.

9. Example: Isolation of a HER4 Ligand

9.1. Materials and Methods

9.1.1. Cell Differentiation Assay

For the identification of ligands specific for HER2, HER3 or HER4, the receptor expression profile of MDA-MB-453 cells offers an excellent indicator for morphologic differentiation inducing activity. This cell line is known to express HER2 and HER3, but contains no detectable EGFR. The results of the semi-quantitative PCR assays (Table III) indicated high level expression of HER4 in MDA-MB-453 cells. In addition, cDNA encoding the prototype HER4 polypeptide of the invention was first isolated from this cell line (Section 6., *supra*).

MDA-MB-453 cells (7500/well) were grown in 50 ml DMEM supplemented with 5% FBS and 1x essential amino acids. Cells were allowed to adhere to 96-well plates for 24 hr. Samples were diluted in the above medium, added to the cell monolayer in 50 ml final volume, and the incubation continued for an additional 3 days. Cells were then examined by inverted light microscopy for morphologic changes.

9.1.2. Source Cells

Serum free media from a panel of cultures of human cancer cells were screened for growth regulatory activity on MDA-MB-453 cells. A human hepatocarcinoma cell line, HepG2, was identified as a source of a factor which induced dramatic morphologic differentiation of the MDA-MB-453 cells.

9.1.3. Purification of HER4 Ligand

The cell differentiation assay described in Section 10.1.1., *supra*, was used throughout the purification procedure to monitor the column fractions that induce morphological changes in MDA-MB-453 cells. For large-scale production of conditioned medium, HepG2 cells were cultured in DMEM containing 10% fetal bovine serum using Nunc cell factories. At about 70% confluence, cells were washed then incubated with serum-free DMEM. Conditioned medium (HepG2-CM) was collected 3 days later, and fresh serum-free medium added to the cells. Two additional harvests of HepG2-CM were collected per cell factory. The medium was centrifuged and stored at -20° C in the presence of 500 mM PMSF.

Ten litres of HepG2-CM were concentrated 16-fold using an Amicon ultrafiltration unit (10,000 molecular weight cutoff membrane), and subjected to sequential precipitation with 20% and 60% ammonium sulfate. After centrifugation at 15,000 x g, the supernatant was extensively dialyzed against PBS and passed through a DEAE-sepharose (Pharmacia) column pre-equilibrated with PBS. The flow-through fraction was then applied onto a 4 ml heparin-acrylic (Bio-Rad) column equilibrated with PBS. Differentiation inducing activity eluted from the heparin column between 0.4 and 0.8 M NaCl. Active heparin fractions

were pooled, brought to 2.0 M ammonium sulfate, centrifuged at 12,000 x g for 5 min, and the resulting supernatant was loaded onto a phenyl-5PW column (8 x 75 mm, Waters). Bound proteins were eluted with a decreasing gradient from 2.0 M ammonium sulfate in 0.1 M Na_2HPO_4, pH 7.4 to 0.1 M Na_2HPO_4. Dialyzed fractions were assayed for tyrosine phosphorylation of MDA-MB-453 cells, essentially as described (Wen *et al.*, 1992, Cell 69:559-72), except PY20 was used as the primary antibody and horseradish peroxidase-conjugated goat F(ab')2 anti-mouse Ig (Cappell) and chemiluminescence were used for detection. Phosphorylation signals were analyzed using the Molecular Dynamics personal densitometer.

9.2. Results

Semi-purified HepG2-derived factor demonstrated a capacity to induce differentiation in MDA-MB-453 cells (FIG. 11, Panel 1-3). With reference to the micrographs shown in FIG. 11, Panel 1-3, untreated MDA-MB-453 cells are moderately adherent and show a rounded morphology (FIG. 11, Panel 1). In contrast, the addition of semi-purified HepG2-derived factor induces these cells to display a noticeably flattened morphology with larger nuclei and increased cytoplasm (FIG. 11, Panel 2 and 3). This HepG2-derived factor preparation also binds to heparin, a property which was utilized for purifying the activity.

On further purification, the HepG2-derived factor was found to elute from a phenyl hydrophobic interaction column at 1.0M ammonium sulfate (fractions 16 to 18). FIG. 11, Panel 4, shows the phenyl column elution profile. Tyrosine phosphorylation assays of the phenyl column fractions revealed that the same fractions found to induce

differentiation of the human breast carcinoma cells are also able to stimulate tyrosine phosphorylation of a 185 kDa protein in MDA-MB-453 cells (FIG. 11, Panel 5). In particular, fraction 16 induced a 4.5-fold increase in the phosphorylation signal compared to the baseline signal observed in unstimulated cells, as determined by densitometry analysis (FIG. 11, Panel 6).

The phenyl fractions were also tested against the panel of cell lines which each overexpress a single member of the EGFR-family (Section 9.1., *supra*). Fraction 17 induced a significant and specific activation of the HER4 kinase (FIG. 10, Panel 1, lane 4) without directly affecting the phosphorylation of HER2, EGFR, or HER3 (FIG. 10, Panel 1-4, lane 4). Adjacent fraction 14 was used as a control and had no effect on the phosphorylation of any of the EGFR-family receptors (FIG. 10, Panel 1-4, lane 5). Further purification and analysis of the factor present in fraction 17 indicates that it is a glycoprotein of 40 to 45 kDa, approximately the same size as NDF and HRG. The HepG2-derived factor also has functional properties similar to NDF and HRG, inasmuch as it stimulates tyrosine phosphorylation of HER2/p185 in MDA-MB-453 cells, but not EGFR in NR5 cells, and induces morphologic differentiation of HER2 overexpressing human breast cancer cells.

Recently, several groups have reported the identification of specific ligands for HER2 (see Section 2., *supra*., including NDF and HRG-α. In contrast to these molecules, the HepG2-derived factor described herein failed to stimulate phosphorylation of HER2 in CHO/HER2 cells, but did stimulate phosphorylation of HER4 in CHO/HER4 cells. These findings are intriguing in view of the ability of the

HepG2-derived factor to stimulate phosphorylation of MDA-MD-453 cells, a cell line known to overexpress HER2 and HER3 and the source from which HER4 was cloned. Since EGFR and HER2 have been shown to act synergistically, it is conceivable that HER4 may also interact with other EGFR-family members. In this connection, these results suggest that NDF may bind to HER4 in MDA-MB-453 cells resulting in the activation of HER2. The results described in Section 10., immediately below, provide evidence that NDF interacts directly with HER4, resulting in activation of HER2.

10. Example: Recombinant NDF-Induced, HER4 Mediated Phosphorylation of HER2

Recombinant NDF was expressed in COS cells and tested for its activity on HER4 in an assay system essentially devoid of other known members of the EGFR-family, notably EGFR and HER2.

A full length rat NDF cDNA was isolated from normal rat kidney RNA and inserted into a cDM8-based expression vector to generate cNDF1.6. This construct was transiently expressed in COS cells, and conditioned cell supernatants were tested for NDF activity using the tyrosine kinase stimulation assay described in Section 8.2., *supra*. Supernatants from cNDF1.6 transfected cells upregulated tyrosine phosphorylation in MDA-MB-453 cells relative to mock transfected COS media FIG. 12, Panel 1. Phosphorylation peaked 10-15 minutes after addition on NDF.

The crude NDF supernatants were also tested for the ability to phosphorylate EGFR (NR5 cells), HER2 (CHO/HER2 1-2500 cells), and HER4 (CHO/HER4 21-2 cells). The NDF preparation had no effect on phosphorylation of EGFR, or HER2 containing cells, but induced a 2.4 to 4 fold increase in tyrosine

phosphorylation of HER4 after 15 minutes incubation (see FIG. 12, Panel 2). These findings provide preliminary evidence that NDF/HRG-α mediate their effects not through direct binding to HER2, but instead by means of a direct interaction with HER4. In cell lines expressing both HER2 and HER4, such as MDA-MB-453 cells and other breast carcinoma cells, binding of NDF to HER4 may stimulate HER2 either by heterodimer formation of these two related transmembrane receptors, or by intracellular crosstalk. Formal proof of the direct interaction between NDF and HER4 will require crosslinking of ^{125}I-NDF to CHO/HER4 cells and a detailed analysis of its binding characteristics.

11. Example: Chromosomal Mapping of the HER4 Gene

A HER4 cDNA probe corresponding to the 5′ portion of the gene (nucleotide positions 34-1303) was used for in situ hybridization mapping of the HER4 gene. In situ hybridization to metaphase chromosomes from lymphocytes of two normal male donors was conducted using the HER4 probe labeled with ^{3}H to a specific activity of 2.6 x 10^{7} cpm/μg as described (Marth *et al.*, 1986, Proc. Natl. Acad. Sci. U.S.A. 83:7400-04). The final probe concentration was 0.05 μg/μl of hybridization mixture. Slides were exposed for one month. Chromosomes were identified by Q banding.

11.1. Results

A total of 58 metaphase cells with autoradiographic grains were examined. Of the 124 hybridization sites scored, 38 (31%) were located on the distal portion of the long arm of chromosome 2 (FIG. 13). The greatest number of grains (21 grains) was located at band q33, with significant numbers of

grains on bands q34 (10 grains) and q35 (7 grains). No significant hybridization on other human chromosomes was detected.

12. Example: Activation of the HER4 Receptor is Involved in Signal Transduction by Heregulin

12.1. Recombinant Heregulin Induction of Tyrosine Phosphorylation of HER4

12.1.1 Materials and Methods

CHO cells expressing recombinant HER4 or HER2 were generated as previously described in Section 8. Cells (1 x 10^5 of CHO/HER2 and CHO/HER4, and 5 x 10^5 of MDA-MB453) were seeded in 24 well plates and cultured 24 h. Cells were starved in serum free media for 1-6 h prior to addition of conditioned media from transfected COS cells, or 25 μg/ml HER2-stimulatory Mab (N28 and N29) (Stancovski *et al.*, 1991, Proc. Natl. Acad. Sci. U.S.A. 88:8691-8695). Following 10 min treatment at room temperature, cells were solubilized (Section 13, *infra*) and immunoprecipitated with 2 μg anti-phosphotyrosine Mab (PY20, ICN Biochemicals) or anti-HER2 Mab (c-neu Ab-2, Oncogene Sciences) and anti-mouse IgG-agarose (Sigma). Western blots were performed using PY20 as described *supra*, and bands were detected on a Molecular Dynamics phosphorimager.

Recombinant rat heregulin was produced as follows. A 1.6 kb fragment encoding the entire open reading frame of rat heregulin (and 324 bp of 5'-untranslated sequence) was obtained by PCR using normal rat kidney RNA as a template. This fragment was inserted into a CDM8-based expression vector (Invitrogen) to generate cNDF1.6. The expression plasmid was introduced into COS-1 cells using the DEAE-dextranchloroquine method (Seed *et al.*, Proc. Natl. Acad. Sci. U.S.A. 1987, 84:3365-3369). After

two days of growth in Dulbecco's Modified Eagle Medium (DMEM)/10% FBS, the medium was replaced with DMEM and the incubation continued for an additional 48 h. Clarified conditioned medium was either used directly or was dialyzed against 0.1 M acetic acid for 2 days, dried, and resuspended as a 20-fold concentrate in DMEM.

12.1.2. HER Tyrosine Phosphorylation

As shown in FIG. 15, recombinant heregulin induces tyrosine phosphorylation of HER4. Tyrosine phosphorylated receptors were detected by Western blotting with an anti-phosphotyrosine Mab a, Monolayers of MDA-MB453 or CHO/HER4 cells were incubated with media from COS-1 cells transfected with a rat heregulin expression plasmid (HRG), or with a cDM8 vector control (-). The media was either applied directly (1x) or after concentrating 20-fold (20x, and vector control). Solubilized cells were immunoprecipitated with anti-phosphotyrosine Mab. b, Monolayers of CHO/HER2 cells were incubated as above with transfected Cos-1 cell supernatants or with two stimulatory Mabs to HER2 (Mab 28 and 29). Solubilized cells were immunoprecipitated with anti-HER2 Mab. Arrows indicate the HER2 and HER4 proteins.

12.1.3. Results

In order to determine if HER4 is involved in signaling by heregulin, the ability of recombinant rat heregulin to stimulate tyrosine phosphorylation in a panel of Chinese hamster ovary (CHO) cells that ectopically express human HER2 or HER4 was examined. The activity of recombinant heregulin was first confirmed by its ability to stimulate differentiation of human breast cancer cells (data not shown) and to

induce tyrosine phosphorylation of a high molecular weight protein in MDA-MB453 cells (FIG. 15, Panel 1). Heregulin had no effect on CHO cells expressing only HER2 (FIG. 15, Panel 3), yet these cells were shown to have a functional receptor since their tyrosine kinase activity could be stimulated by either of two antibodies specific to the extracellular domain of HER2 (FIG. 15, Panel 3). However, heregulin was able to induce tyrosine phosphorylation of a 180K protein in CHO cells expressing HER4 (FIG. 15, Panel 2).

Species differences in ligand-receptor interactions have been reported for EGF receptor (Lax *et al.*, 1988, Mol. Cell. Biol. 8:1970-1978). It is unlikely that such differences are responsible for our failure to detect a direct interaction between rat heregulin and human HER2, since previous studies have shown that rat heregulin does not directly interact with rat HER2/neu (Peles *et al.*, *supra*). In addition, rat, rabbit, and human heregulin share high sequence homology and have been shown to induce tyrosine phosphorylation in their target cells of human origin (Wen D. *et al.*, *supra*; Holmes *et al.*, *supra*; and Falls *et al.*, *supra*).

12.2. Expression of Recombinant HER2 and HER4 in Human CEM Cells

12.2.1. Materials and Methods

cNHER2 and cNHER4 expression plasmids were generated by insertion of the complete coding sequences of human HER2 and HER4 into cNEO, an expression vector that contains an SV2-NEO expression unit inserted at a unique BamHI site of CDM8. These constructs were linearized and transfected into CEM cells by electroporation with a Bio-Rad Gene Pulser apparatus essentially as previously described (Wen *et al.*, *supra*). Stable clones were selected in RPMI/10%

FBS supplemented with 500 μg/ml active Geneticin. HER2 immunoprecipitations were as described in FIG. 15, using 5 x 10^6 cells per reaction, and the HER2 Western blots were performed with a second anti-HER2 Mab (c-neu Ab-3, Oncogence Sciences). For metabolic labeling of HER4, 5 x 10^6 cells were incubated for 4-6 h in methionine and cysteine-free Minimal Essential Medium (MEM) supplemented with 2% FBS and 250 μCi/ml [^{35}S]Express protein labeling mix (New England Nuclear). Cells were washed twice in RPMI and solubilized as above. Lysates were then incubated for 6 h, 4° C with 3 μl each of two rabbit antisera raised against synthetic peptides corresponding to two regions of the cytoplasmic domain of human HER4 (^{864}LARLLEGDEKEYNADGG^{88} [SEQ ID No:31] and ^{1010}EEDLEDMMDAEEY^{1022} [SEQ ID No:32]). Immune complexes were precipitated with 5 μg goat anti-rabbit Ig (Cappel) and Protein G Sepharose (Pharmacia). Proteins were resolved on 7% SDS-polyacrylamide gels and exposed on the phosphorimager. For Mab-stimulation assays, 5 x 10^6 cells were resuspended in 100 μl RPMI and 25 μg/ml Mab was added for 15 min at room temperature. Control Mab 18.4 is a murine IgG_1 specific to human amphiregulin (Plowman *et al.*, 1990, Mol. Cell. Biol. 10:1969-1981). Following Mab-treatment, cells were washed in PBS, solubilized (Section 13, *infra*), and immunoprecipitated with anti-HER2 Mab (Ab-2). Tyrosine phosphorylated HER2 was detected by PY20 Western blot as in FIG. 15.

12.2.2. Expression of HER2 and HER4 in Human CEM Cells

Expression of recombinant HER2 and HER4 in human CEM cells is shown in FIG. 16. Transfected CEM cells were selected that stably express either HER2, HER4, or both recombinant receptors. In FIG. 16, Panel 1,

recombinant HER2 was detected by immunmoprecipitation of cell lysates with anti-HER2 Mab (Ab-2) and Western blotting with another anti-HER2 Mab (Ab-3). In FIG. 16, Panel 2, recombinant HER4 was detected by immunoprecipitation of ^{35}S-labeled cell lysates with HER4-specific rabbit anti-peptide antisera. In FIG. 16, Panel 3, three CEM cell lines were selected that express one or both recombinant receptors and aliquots of each were incubated with media control (-), with two HER2-stimulatory Mabs (Mab 28 and 29), or with an isotype matched control Mab (18.4). Solubilized cells were immunoprecipitated with anti-HER2 Mab (Ab-2) and tyrosine phosphorylated HER2 was detected by Western blotting with an anti-phosphotyrosine Mab. The size in kilodaltons of prestained high molecular weight markers (Bio-Rad) is shown on the left and arrows indicate the HER2 and HER4 proteins.

12.2.3. Results

These findings of Example 12 support the earlier observation that HER2 alone is not sufficient to transduce the heregulin signal. To further address this possibility, a panel of human CEM cells that express the recombinant receptors either alone or in combination was established. The desired model system was of human origin, since many of the reagents against erbB family members are specific to the human homologues. CEM cells are a human T lymphoblastoid cell line and were found to lack expression of EGF receptor, HER2, HER3, or HER4, by a variety of immunologic, biologic, and genetic analyses (data not shown). FIG. 16 demonstrates the selection of three CEM cell lines that express only HER2 (CEM 1-3), only HER4 (CEM 3-13), or both HER2 and HER4 (CEM 2-9). The presence of a functionally and structurally intact

HER2 in the appropriate cells was confirmed by the induction of HER2 tyrosine phosphorylation by each of the two antibodies specific to the extracellular domain of HER2, but not by an isotype matched control antibody (FIG. 16, Panel 3).

12.3. Heregulin Induction of Tyrosine Phosphorylation in CEM Cells Expressing HER4

12.3.1. Materials and Methods

Recombinant rat heregulin was prepared as in FIG. 15, and diluted to 7x in RPMI. The HER4-specific Mab was prepared by immunization of mice with recombinant HER4 (manuscript in preparation). CEM cells (5×10^6) were treated with the concentrated supernatants for 10 min, room temperature and precipitated with PY20 or anti-HER2 Mab (Ab-2) as described in FIG. 15. Immunoprecipitation with anti-HER4 Mab was performed by incubation of cells lysates with a 1:5 dilution of hybridoma supernatent for several hours followed by 2 μg rabbit anti-mouse Ig (cappel) and Protein A Sepharose CL-4B (Pharmacia). PY20 Westerns as described in FIG. 15.

12.3.2. Heregulin Induction of Tyrosine Phosphorylation in CEM Cells Expressing HER4

As shown in FIG. 17, heregulin induces tyrosine phosphorylation in CEM cells expressing HER4. Three CEM cell lines that express either HER2 or HER4 alone (CEM 1-3 and CEM 3-13) or together (CEM 2-9) were incubated with 7x concentrated supernatants from mock- (-) or heregulin-transfected (+) COS-1 cells. Solubilized cells were immunoprecipitated (IP) with anti-phosphotyrosine Mab (PY20) (FIG. 17, Panel 1); HER2-specific anti-HER2 Mab (Ab-2) (FIG. 17, Panel 2); or HER4-specific Mab (6-4) (FIG. 17, Panel 3). In

each case, tyrosine phosphorylated receptors were detected by Western blotting with anti-phosphotyrosine Mab. The size in kilodaltons of prestained molecular weight markers (BioRad) is shown on the left and arrows indicate the HER2 and HER4 proteins.

12.3.3 Results

The panel of CEM cells were then analyzed by phosphotyrosine Western blots of cells lysates following treatment with heregulin and immunoprecipitation with three different monoclonal antibodies (Mabs). Precipitation with an anti-phosphotyrosine antibody (PY20) again demonstrates that heregulin is able to stimulate tyrosine phosphorylation in cells expressing HER4, but not in cells expressing only HER2 (FIG. 17, Panel 1). However, precipitation with an antibody specific to the extracellular domain of HER2 demonstrates that HER2 is tyrosine phosphorylated in response to heregulin in cells that co-express HER4 (FIG. 17, Panel 2). Furthermore, precipitation with a HER4-specific Mab confirms that heregulin induces tyrosine phosphorylation of HER4 irrespective of HER2 expression (FIG. 17, Panel 3). Due to co-expression of HER2 and HER4 in many breast carcinomas, these findings suggest that earlier studies of heregulin-HER2 interactions may require reevaluation.

12.4. Covalent Cross-linking of Iodinated Heregulin to HER4

12.4.1. Materials and Methods

To facilitate purification, recombinant heregulin was produced as an epitope-tagged fusion with amphiregulin. The 63 amino acid EGF-structural motif of rat heregulin (Wen *et al.*, *supra*) from serine 177 to tyrosine 239 was fused to the N-terminal 141 amino

acids of the human amphiregulin precursor (Plowman *et al., supra*). This truncated portion of heregulin has previously been shown to be active when expressed in *E. coli* (Holmes *et al., supra*), and the N-terminal residues of amphiregulin provide an epitope for immunologic detection and purification of the recombinant protein. This cDNA fragment was spliced into a cDM8 based expression vector for transient expression in COS-1 cells. Recombinant heregulin was purified by anion exchange and reverse phase chromatography as shown to be active based on the specific stimulation of HER4 tyrosine phosphorylation. Purified heregulin was iodinated with 250 μCi of ^{125}I-labeled Bolton-Hunter reagent (NEN). CHO/HER4 or CHO/HER2 cells were incubated with ^{125}I-heregulin (10^5-cpm) for 2 h at 4° C. Monolayers were washed in PBS and 3 mM Bis(sulfosuccinimidyl) suberate (BS^3, Pierce) was added for 30 min on ice. The cells were washed in tris-buffered saline, dissolved in SDS sample buffer, run on a 7% polyacrylamide gel, and visualized on the phosphorimager.

12.4.2. Results

As shown in FIG. 18, previous binding and covalent cross-linking studies have demonstrated that p45 binds specifically to HER4 and displays a single high-affinity site with a K_d of 5 nM on CHO/HER4 cells (Section 13, *infra*). Preliminary cross-linking studies have been performed on these cells with recombinant heregulin revealing a high molecular weight species that corresponds to the heregulin-HER4 receptor complex.

12.5 Results

As the data demonstrate heregulin induces tyrosine phosphorylation of HER4 in the absence of HER2. In contrast, heregulin does not directly stimulate HER2. However, in the presence of HER4, heregulin induces phosphorylation of HER2, presumably either by transphosphorylation or through receptor heterodimerization. Together, these experiments suggest that HER4 is the receptor for heregulin.

Most breast cancer cells that overexpress HER2 have been shown to be responsive to heregulin, whereas HER2-positive ovarian and fibroblast lines do not respond to the ligand. This observation could be explained by the fact that HER4 is co-expressed with HER2 in most or all of the breast cancer cell lines studied, but not in the ovarian carcinomas. Furthermore, overexpression of HER2 in heregulin-responsive breast cancer cells leads to increased binding, whereas expression of HER2 in heregulin-unresponsive ovarian or fibroblast cells has no effect (Peles *et al.*, *supra*).

Northern and in situ hybridization analyses localizes HER4 to the white matter and glial cells of the central and peripheral nervous system, as well as to cardiac, skeletal, and smooth muscle. This distribution is consistent with HER4 being involved in signaling by the neurotropic factors, GGF, and ARIA. Recognition of HER4 as a primary component of the heregulin signal transduction pathway will assist in deciphering the molecular mechanisms that results in its diverse biologic effects.

13. Example: Purification of the HER4 ligand, p45

13.1 Materials and Methods

13.1.1. Cell Culture and Reagents

MDA-MB 453 cells were obtained from the American Type Culture Collection (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and amino acids (Life Technologies, Inc.). HepG2 cells were obtained from Dr. S. Radka and cultured in 10% fetal bovine serum containing DMEM. For large scale production of serum-free conditioned medium, HepG2 cells were propagated in Nunc cell factories. Chinese hamster ovary cells (CHO-KI) expressing high levels of either recombinant human $p185^{erbB2}$ (CHO/HER2) or recombinant human $p180^{erbB4}$ (CHO/HER4) were generated and cultured as described in Section 8. N29 monoclonal antibody to the extracellular portion of the human HER2 receptor was a gift from Dr. Y. Yarden. Ab-3 c-*neu* monoclonal antibody that reacts with the human $p185^{erbB2}$ was from Oncogene Science Inc.

13.1.2. Human Breast Cancer Cell Differentiation Assay

MDA-MB-453 human breast cancer cells overexpress $p185^{erbB2}$ but do not express the EGFR at their surface (Kraus, 1987, EMBO J. 6:605-610). A cell differentiation assay was used to monitor the chromatography fractions for their ability to induce phenotypic differentiation in MDA-MB-453 cells.

13.1.3. Purification of p45

Medium conditioned by HepG2 cells (HepG2-CM, 60 liters) was concentrated 26-fold using an Amicon ultrafiltration unit (10,000 molecular weight cutoff membranes) and then subjected to 50% ammonium sulfate ($(NH_4)_2SO4_4$) precipitation. After centrifugation at

25,000 x *g* for 1 h, the supernatant was loaded, as five separate runs, on a phenyl-Sepharose column (2.5 x 24.5 cm, Pharmacia LKB Biotechnology Inc.) equilibrated with 1.9 M $(NH_4)_2SO_4$ in 0.1 M Na_2HPO_4, pH 7.4. Bound proteins were eluted with a 240 ml linear decreasing gradient from 1.9 M to 0 M $(NH_4)_2SO_4$ in 0.1 M phosphate buffer, pH 7.4. The flow rate was 70 ml/h, and 5.8-ml fractions were collected. Active fractions were pooled, concentrated, dialyzed against PBS, and then applied (three separate runs) to a DEAE-Sepharose column (2.5 x 25 cm, Pharmacia) equilibrated with PBS, pH 7.3. The flow rate was 1 ml/min. The column flow-through was then loaded (two separate runs) on a CM-Sepharose Fast Flow column (2.5 x 13.5 cm, Pharmacia) pre-equilibrated with PBS, pH 7.3. Proteins were eluted at 1 ml/min. with a 330-ml gradient from PBS to 1 M NaCl in PBS. Fractions of 5 ml were collected. The active material was loaded on a TSKgel heparin-5PW HPLC column (7.5 x 75 mm, TosoHaas) equilibrated with PBS. The flow rate was 0.5 ml/min. A 50-ml linear NaCl gradient (PBS to 2 M in PBS) followed by an isocratic elution with 2 M NaCl was used to elute the bound proteins. Fractions of 1 ml were collected. Active fractions corresponding to the 1.3 M NaCl peak of protein were pooled and concentrated. A Protein Pak SW-200 size exclusion chromatography column (8 x 300 mm, Waters) equilibrated with 100 mM Na_2HPO_4, pH7.4, 0.01% Tween 20 was used as a final step of purification. The flow rate was 0.5 ml/min., and 250-μl fractions were collected. Column fractions were then analyzed by SDS-PAGE (12.5% gel) under reducing conditions and proteins detected by silver staining.

13.1.4. Detection of Tyrosine-Phosphorylated Proteins by Western Blotting

Aliquots of PBS-dialyzed column fractions were diluted to 200 µl in PBS, then added to individual wells of 48-well plated containing either 5 x 10^5 MDA-MB-453 cells, 2 x 10^4 CHO/HER2 cells or 5 x 10^4 CHO/HER2 cells. Following a 10-min. incubation at 37° C, cells were washed and then lysed in 100 µl of boiling electrophoresis sample buffer. Lysates were heated at 100° C for 5 min., cleared by centrifugation, and then subjected to SDS-PAGE. After electrophoresis, proteins were transferred to nitrocellulose. The membrane was blocked for 2 h at room temperature with 6% hovine serum albumin in 10 mM Tria-HC1, pH 8.0, 150 mM NaC1, 0.05% Tween 20. PY20 monoclonal anti-phosphotyrosine antibody (ICN, 2 h at 22° C) and horseradish peroxidase-conjugated goat anti-mouse IgG $F(ab')_2$ (Cappel, 1h at 22° C) were used as primary and secondary probing reagents, respectively. Proteins phosphorylated on tyrosine residues were detected with a chemiluminescence reagent (Amersham Corp.).

13.1.5. CHO/HER2 Stimulation Assay

CHO/HER2 cells were seeded in 24-well plates at 1 x 10^5 cells/well and cultured 24 h. Monoclonal antibody N29 specific to the extracellular domain of $p185^{erbB2}$ (Stancovski *et al.*, 1991, PNAS 88:8691-8695) was added at 25 µg/ml. Following a 20-min. incubation at room temperature, media were removed and cells were solubilized for 10 min. on ice in PBS-TDS (10 mM sodium phosphate, pH 7.25, 150 mM NaC1, 1% Triton, 0.5% sodium deoxycholate, 0.1% SDS, 0.2% NaN_3, 1 mM NaF, 1 m M phenylmethylsulfonyl fluoride, 20 µg/ml aprotinin) with occasional vortexing. Clarified

extracts were incubated for 2 h at 4° C with an antip-185^{erbB2} antibody (Ab-3 c-*neu*, Oncogene Science Inc.). Rabbit anti-mouse IgG (Cappel) and protein A-Sepharose were then added, and samples were incubated an additional 30 min. Immune complexes were washed 3 times with PBS-TDS, resolved on a 7% polyacrylamide gel, and electrophoretically transferred to nitrocellulose. Phosphorylation of the receptor was assessed by Western blot using a 1:1000 dilution of PY20 phosphotyrosine primary antibody (ICN Biochemicals) and a 1:500 dilution of ^{125}I-sheep anti-mouse F(ab')$_2$(Amersham Corp.).

13.1.6. Covalent Cross-linking of Iodinated p45

HPLC-purified p45 (1.5 μg) was iodinated with 250 μCi of ^{124}I-labeled Bolton-Hunter reagent obtained from Du Pont-New England Nuclear. ^{125}I-p45 was purified by filtration through a Pharmacia PD-10 column. The specific activity was 10^4 cpm/ng. ^{125}I-p45 retained its biological activity as confirmed in a differentiation assay as well as a kinase stimulation assay (data not shown). Binding of radiolabeled p45 was performed on 2 x 10^5 CHO/HER4 cells and 4 x 10^5 CHO-KI or CHO/HER2 cells in 12-well plates. Cell monolayers were washed twice with 1 ml of ice-cold binding buffer (DMEM supplemented with 44 mM sodium bicarbonate, 50 mM BES [N-, N-Bis (2-hydroxyethyl) -2-aminoethan-sulfonic acid], pH 7.0, 0.1% bovine serum albumin) and then incubated on ice for 2 h with 50 ng/ml ^{125}I-p45 in the absence or the presence of 250 ng/ml unlabeled p45. The monolayers were washed twice with PBS and then incubated in the presence of 1 mM *bis*(sulfosuccinimidyl)suberate (BS^3, Pierce) in PBS for 45 min. on ice. Supernatants were discarded, and the reaction was quenched by adding 0.2 M glycine in PBS.

Cells were washed and then lysed by adding 150 μl of boiling electrophoresis sample buffer containing 0.1 M dithiothreitol. Samples were boiled for 5 min. and 50 μl of each sample was loaded on 7.5% polyacrylamide gels. Dried gels were analyzed using a Molecular Dynamics PhosphorImager and then exposed to Kodak X-Omat AR films.

13.1.7. Binding Analysis of Iodinated p45

CHO/HER4 cells, CHO-KI cells (10^5 cells/well), and CHO/HER2 cells (2 x 10^5 cells/well) were seeded in 24-well plates. After 48 h, cells were washed with binding buffer and then incubated with increasing concentrations of ^{125}I-p45. Nonspecific binding was determined in the presence of excess unlabeled p45. After a 2-h incubation at 4° C, the cells were washed three times with binding buffer and then lysed in 500 μl of 0.5M NaOH, 0.1% SDS. Cell-associated radioactivity was determined by using a γ-counter. Scatchard analysis was performed using the computerized LIGAND program (Munson and Rodbard, 1980, Anal. Biochem 107:220-239).

13.1.8. N-terminal Amino Acid Sequence

The N-terminal sequence analysis of p45 (25 pmol) was performed as previously described (Shoyab *et al.*, 1990, Proc. Natl. Acad. Sci. 87:7912-7916).

13.2. Purification of the HER4 ligand, p45

Sixty liters of medium conditioned by HEPG2 cells was used as a starting material, and throughout the purification procedure, bioactivity was assessed by a cell differentiation assay described in Section 10.1.1., *supra*. After concentration (1540 mg of protein) and ammonium sulfate precipitation, the

active material (1010 mg of protein) was loaded on a phenyl-Sepharose column (FIG. 19, Panel 1). Column fractions 40-85 (348 mg of protein eluting between 1M ammonium sulfate and 0M ammonium sulfate) were found to induce morphological changes in MDA-MB-453 cells. The biologically active column flow-through (174 mg of protein) was subjected to a cation-exchange chromotography (FIG. 19, Panel 2) with activity eluting between 0.35 and 0.48 M NaCl. The active fractions were pooled (1.5 mg of protein) and applied to an analytical heparin column (FIG. 19, Panel 3). The differentiation activity eluted from the heparin column between 0.97 and 1.45 M NaCl (fractions 27-38). Size exclusion chromatography of the heparin column fractions 35-38 achieved a homogeneous preparation of the human breast cancer cell differentiation factor. A major protein peak eluted with a molecular weight greater than 70,000 (FIG. 19, Panel 4). Fractions 30 and 32 assayed at 30 ng/ml confirmed the bioactivity of this protein with phenotypic changes being apparent after 24 hours. SDS-PAGE analysis of these column fractions followed by silver staining of the gel showed that the biologically active peak contained a single protein migrating around 45 kDA (FIG. 20). The faint 67 kDa band corresponds to a staining artifact, as evidenced by the left lane of the gel, which contained no sample. The amount of pure protein recovered in fractions 30-33 was estimated to be 6 micrograms. The difference in the molecular weight estimated by size exclusion chromatography and SDS-PAGE indicates that this protein may form dimers or oligomers under non-denaturing conditions.

13.3. N-terminal Amino Acid Sequence of p45

Twenty-five pmol of p45 was subjected to direct amino acid sequencing, identifying the sequence Ser-Gly-X-Lys-Pro-X-X-Ala-Ala [SEQ ID No:33]. An X denotes a sequenator cycle in which a precise amino acid could not be assigned. Comparison of this partial sequence with two protein data bases (GenBank release 73, EMBL release 32) revealed a perfect homology between the identified residues and a region of the amino terminus of heregulin (Holmes *et al.*, *supra*) The N-terminal serine residue of p45 corresponds to residue 20 of the deduced amino acid sequence of heregulins.

13.4. p45 Stimulates Protein Phosphorylation

FIG. 21, Panel 1 shows the stimulatory effect of sequential fractions from the size exclusion chromatography column on tyrosine phosphorylation in MDA-MB-453 cells. Densitometric analysis of the autoradiogram revealed that fractions 30-34 were essentially equipotent. Homogeneously purified p45 specifically stimulated tyrosine phosphorylation of $p180^{erbB4}$ (FIG. 21, Panel 2). p45 was not able to stimulate phosphorylation in CHO/HER2 cells, and the cell were found to express functional $p185^{erbB2}$ receptor as evidenced by immunoreactivity with 5 monoclonal antibodies specific to different regions of $p185^{erbB2}$. p45 has an N-terminal amino acid sequence similar to the recently isolated $p185^{erbB2}$ ligand.

13.5. Binding and Covalent Cross-linking of p45 to $p180^{erbB4}$

Binding and cross-linking studies were performed in order to confirm that p45 was able to bind to $p180^{erbB4}$. Binding studies revealed that while no

specific binding of ^{125}I-p45 to CHO-KI and CHO/HER2 cells could be measured, CHO/HER4 cells displayed a single high affinity site (Kd about 5nM) with 7 x 104 receptors/cell (FIG. 22, Panel 1). The results of iodinated p45 cross-linking to CHO-KI, CHO/HER2, or CHO/HER4 cells are presented in FIG. 22, Panel 2. Whereas no cross-linked species was observed in either CHO-KI or CHO/HER2 cells, four distinct bands were observed in CHO/HER4 cells, migrating as 45-, 100-, and 210-kDa species, and a very high molecular weight species. In the presence of unlabeled p45, ^{125}I-p45 binding was greatly reduced. The 45 kDa band represents uncross-linked yet p180^{erbB4} associated ^{125}I-p45. The 210 kDa band corresponds to the p45-p180^{erbB4} complex (assuming an equimolar stoichiometry of ligand and receptor), whereas the high molecular weight band is presumed to be a dimerized form of the receptor-ligand complex. The 100 kDa band could represent a truncated portion of the extracellular domain of the p180^{erbB4} receptor complexed to ^{125}I-p45 or a covalently associated p45 dimer. The c-kit ligand provides precedence for cross-linked dimers (Williams *et al.*, 1990, Cell 63:167-174).

13.6. Results

The HER4 ligand, p45, purified from medium conditioned by HepG2, induces differentiation of breast cancer cells and activates tyrosine phosphorylation of a 185 kDa protein in MDA-MB-453 cells. p45 is not capable of directly binding to p185^{erbB2} but shows specificity to HER4/p180^{erbB4}.

14. Example: Targeted Cytotoxicity Mediated By A Chimeric Heregulin-Toxin Protein

14.1. Materials and Methods

14.1.1. Reagents and Cell Lines

Heregulin β2-Ig and the mouse monoclonal antibody directed against the *Pseudomonas* exotoxin (PE) was supplied by Dr. J.-M. Colusco and by Dr. Tony Siadek, respectively (Bristol-Myers-Squibb, Seattle, WA). The cell lines BT474, MDA-MB-453, T47D, SKBR-3, and MCF-7 (all breast carcinoma), LNCaP (prostate carcinoma), CEM (T-cell leukemia) and SKOV3 (ovarian carcinoma) were obtained from ATCC (Rockville, MD). The H3396 breast carcinoma cell line and the L2987 lung carcinoma cell line were established at Bristol-Myers-Squibb (Seattle, WA). The AU565 breast carcinoma cell line was purchased from the Cell Culture laboratory, Naval Biosciences Laboratory (Naval Supply Center, Oakland, CA). All cell lines were of human origin. BT474 and T47D cells were cultured in IMDM supplemented with 10% fetal bovine serum (FBS) and 10 μg/ml insulin. MCF-7, H3396, LNCaP and L2987 were cultured in IMDM supplemented with 10% FBS. SKBR3 and SKOV3 cells were grown in McCoys media supplemented with 10% FBS and 0.5% non-essential amino acids. AU565 cells were cultured in RPMI 1640 media supplemented with 15% FBS and CEM transfectants (see section 15.1.5., *infra*) were cultured in RPMI 1640 supplemented with 10% FBS and 500 μg/ml G418.

14.1.2. Construction of HAR-TX β2 Expression Plasmid

Rat heregulin cDNA (Wen *et al.*, 1994, Mol. Cell. Biol. 14:1909-1919) was isolated by RT-PCR using mRNA from rat kidney cells as template. The cDNA was

prepared in chimeric form with the AR leader sequence by a two-step PCR insertional cloning protocol using cARP (Plowman *et al.*, 1990, Mol. Cell. Biol. 10:1969-1981) as template to amplify the 5′ end of the chimeric ligand using the oligonucleotide primers

CARP5:

(5′-CGGAAGCTTCTAGAGATCCCTCGAC-3′) [SEQ ID No:34]

and

ANSHLIK2:

(3′CCGCACACTTTATGTGTTGGCTTGTGTTTCTTCTATTTTTTCCA TTTTTG-5′) [SEQ ID No:35].

The EGF-like domain PCR was amplified from cNDF1.6 (Plowman *et al.*, 1993, Nature 366:473-475) using the oligonucleotide primers

ANSHLIK1:

(5′-CAAAAATGGAAAAAATAGAAGAAACAGAAGCCATCTCATAA AGTGTGCGG-3′) [SEQ ID No:36]

and

XNDF1053:

(3′-GTCTCTAGATTAGTAGAGTTCCTCCGCTTTTTCTTG-5′) [SEQ ID No:37].

The products were combined and reamplified using the oligonucleotide primers CARP5 and XNDF1053. The HAR (heregulin-amphiregulin) construct (cNANSHLIK) was PCR amplified in order to insert an Nde I restriction site on the 5′ end and a Hind III restriction site on the 3′ end with the oligonucleotide primers

NARP1:

(5′-GTCAGAGTTCATATGGTAGTTAAGCCCCCCCAAAAC-3′) [SEQ ID No:38]

and

NARP4:

(3′-GGCAGTTCTATGAACACGTTCACGGGCTTGCTTAAATGACCGCTGGCA ACGGTCTTGATACAATACCGTAGAAAAATGTTTAGCCTCCTTGAGATGTTCGAA TCTCCTAGAAAC-5′) [SEQ ID No:39].

The resulting 287 bp DNA fragment was digested with Nde I and Hind III, followed by ligation into the compatibly digested expression plasmid pBW 7.0 which contained, in frame at the 5′ fusion site, the nucleotide sequence encoding for of PE40 (Friedman *et al.*, 1993, Cancer Res. 53:334-339). The resulting expression plasmid pSE 8.4 then contained the gene fusion encoding the chimeric heregulin-toxin protein, under the control of a IPTG-inducible T7 promoter.

14.1.3. Expression and Isolation of Recombinant HAR-TX β2 Protein

The plasmid pSE 8.4 encoding the chimeric protein HAR-TX β2 was transformed into the *E. coli* strain BL21 (λDE3). Cells were grown by fermentation in T broth containing 100 μg/ml ampicillin at 37°C to a optical density of A_{650} = 4.8, followed by induction of protein expression with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). After 90 minutes the cells were harvested by centrifugation. The cell pellet was frozen at -70°C, then thawed and resuspended at 4°C in solubilization buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1 ug/ml leupeptin, 2 ug/ml aprotinin, 1 ug/ml pepstatin-A, 0.5 mM PMSF) containing 1% tergitol by homogenization and sonication. The insoluble material of the suspension, containing inclusion bodies with the HAR-TX β2 protein, was pelleted by centrifugation and washed three times with solubilization buffer containing 0.5% tergitol (first wash), 1 M NaCl (second wash), and buffer alone (third wash).

The resulting pellet containing pre-purified inclusion bodies was dissolved in 6.5 M guanidine-HCl, 0.1 M Tris-HCl (pH 8.0), 5 mM EDTA; sonicated; and refolded by rapid dilution (100-fold) into 0.1 M Tris-HCl (pH 8.0), 1.3 M urea, 5 mM EDTA, 1 mM glutathione,

and 0.1 mM oxidized glutathione at 4°C. The addition of the denaturating agent urea at low concentration was utilized to allow slow refolding and avoid the formation of aggregates. The refolded HAR-TX β2 protein was diluted 2-fold with 50 mM sodium phosphate (pH 7.0) and applied to a cation-exchange resin (POROS 50 HS, PerSeptive Biosystems, Cambridge, MA), pre-equilibrated in the same buffer. The HAR-TX β2 protein was eluted with a 450 nM NaCl step gradient in 50 mM sodium phosphate (pH 7.0) and fractions were analyzed using SDS-PAGE and Coomassie blue staining. Final purification of pooled fractions was performed by chromatography using Source 15S cation-exchange media (Pharmacia, Uppsala, Sweden) equilibrated with 50 mM sodium phosphate (pH 6.0). Chimeric HAR-TX β2 protein was eluted with a gradient of 0-1 M NaCl in the same buffer and analyzed by SDS-PAGE.

14.1.4. ELISA Test for Determination of Binding Activity

Membranes from 5 x 10^7 MDA-MB-453 cells were prepared and coated to 96 well plates as previously described for H3396 human breast carcinoma cells (Siegall *et al.*, 1994, J. Immunol. 152:2377-2384). Subsequently, the membranes were incubated with titrations of either HAR-TX β2 or PE40 ranging from 0.3 - 300 ug/ml and the mouse monoclonal anti-PE antibody EXA2-1H8 as the secondary reagent (Siegall *et al.*, *supra*). The isolate of the toxin portion PE40 alone was used to determine unspecific binding activity to the membrane preparations, in comparison with the specific binding activity of HAR-TX β2.

14.1.5. Phosphotyrosine Analysis of transfected CEM cell lines

CEM cells expressing various receptors of the EGF-R family (1-5 x 10^6 cells) were stimulated with 500 ng/ml HAR-TX β2 for 5 minutes at room temperature. The cells were pelleted and resuspended in 0.1 ml lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1% NP40, 0.5% deoxycholate, 0.1% sodium dodecylsulfate, 1 mM sodium orthovanadate) at 4°C. Insoluble material was pelleted by centrifugation at 10,000 x g for 30 seconds, and samples were analyzed by SDS-PAGE and subsequent Western blot analysis using the anti-phosphotyrosine antibodies 4G10 (ICN, Irvine, CA) and PY20 (Upstate Biotechnology, Lake Placid, New York).

14.1.6. Cytotoxicity Assays

For cytotoxicity assays, tumor cells (10^5 cells/ml) in growth medium were added to 96-well flat bottom tissue culture plates (0.1 ml/well) and incubated at 37°C for 16 h. Cells were incubated with HAR-TX β2 for 48 h at 37°C, washed twice with phosphate buffered saline (PBS), followed by addition of 200 µl/well of 1.5 µM calcein-AM (Molecular Probes Inc., Eugene, OR). The plates were incubated for 40 minutes at room temperature (RT), and the fluorescence measured using a Fluorescence Concentration Analyzer (Baxter Heathcare Corp., Mundelein, IL) at excitation/emission wavelengths of 485/530 nm. Calcein-AM is membrane permeable and virtually non-fluorescent. When it is hydrolyzed by intracellular esterases, an intensely fluorescent product, calcein is formed. The % cytotoxicity was calculated as previously described (Siegall *et al., supra*). To determine the specificity of the cytotoxic effect of HAR-TX β2 competitive assays were performed on LNCaP

and on MDA-MB-453 cells. Treated essentially as described above, plates were incubated with increasing concentrations of HAR-TX β2 in presence heregulin β2-Ig (0.002-5.0 μg/ml) or with HAR-TX β2 (50 ng/ml). Isotype matched L6-Ig (Hellström *et al.*, 1986, Cancer Res. 46:3917-3923) was used as negative control for the competition assay.

14.1.7. Generation of Monoclonal Antibodies to HER4

HER4, expressed in baculovirus, was used as the immunogen for subcutaneous injection into 4-6 week old female BALB/c mice. Immunization was performed 4 times (approximately 1 month apart) with 20 μg of HER4 protein given each time. Spleen cells from immunized mice were removed four days after the final immunization and fused with the mouse myeloma line P2x63-Ag8.653 as previously described (Siegall *et al.*, *supra*). Positive hybridoma supernatants were selected by ELISA screening on plates coated with HER4 transfected CHO cells (Plowman *et al.*, 1993, Nature 366:473-475) and selected against parental CHO cells and human fibroblasts. Secondary screening was performed by ELISA on plates coated with baculovirus/HER4 membranes. Positive hybridomas were rescreened by two additional rounds of ELISA using CHO/HER4 and HER4 negative cells, and identified false positive were removed. Positive hybridomas were cloned in soft agar and tested for reactivity with the HER4 positive MDA-MB-453 human breast carcinoma cell line and CEM cells co-transfected with HER4 and HER2. Anti-HER4 hybridoma line 6-4-11 (IgG1) was cloned in soft agar and screened for reactivity to native and denatured HER4. A second antibody (7-142, IgG2a) was

also selected and found to bind to the cytoplasmic domain of HER4.

The characteristics for both antibodies are summarized in Table VI (see section 15.2.8., *infra*)

14.1.8. Quantitation of HER2, HER3, and HER4 Protein in tumor cell lines

Cell-surface expression of HER2, HER3, and HER4 protein was determined by quantification of specific antibody binding, detected by the CAS Red Chromagen system (Becton Dickson Cellular Imaging System, Elmhurst, IL). HER2 staining was performed by using mouse anti-HER2 mAb 24.7 (Stancovski *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8691-8695) as primary, and biotinylated goat anti-mouse IgG (Jackson Labs, West Grove, PA) as secondary antibody as previously described (Bacus *et al.*, 1993, Cancer Res. 53:5251-5261). For detection of HER3 and HER4 the primary antibodies used were, respectively, mouse anti-HER3 mAb RTJ2 (Santa Cruz Biotech, Santa Cruz, CA) at 2.5 µg/ml concentration or mouse anti-HER4 mAb 6-4-11 at 15 µg/ml concentration followed by incubation with biotinylated rabbit anti-mouse IgG (Zymed Labs, South San Francisco, CA).

The staining procedure was performed at RT as follows: cells were fixed in 10% neutral buffered formalin for 60 minutes, washed with H_2O and rinsed with Tris buffered saline (TBS; 0.05 M Tris, 0.15 M NaCl, pH 7.6). Unspecific binding sites were blocked by incubation with 10% goat serum (for HER2) or rabbit serum (for HER3 and HER4) in 0.1% bovine serum albumin/TBS for 15 minutes. Subsequently, cells were incubated with primary and secondary antibodies for 30 and 20 minutes, respectively, followed by incubation with alkaline phosphatase conjugated streptavidin

(Jackson Labs) for 15 minutes, with TBS washing between the steps. Detection of antibody binding was achieved using CAS Red Chromagen (Becton Dickinson Cellular Imaging System, supra) for 4 minutes (HER2), 8-10 minutes (HER3), and 10-12 minutes (HER4). Cells were counterstained as described in the CAS DNA stain protocol (Becton Dickinson Cellular Imaging System).

14.1.9. Image Analysis

Image analysis was performed as previously described (Bacus *et al.*, 1993, *supra*; Bacus *et al.*, 1992, Cancer Res. 52:2580-2589; Peles *et al.*, 1992, Cell 69:205-216). In the quantitation of HER2, both solid state imaging channels of the CAS 200 Image Analyzer (Becton Dickinson Cellular Imaging System), a microscope-based, two-color system were used. The two imaging channels were specifically matched to the two components of the stains used. One channel was used for quantitating the total DNA of the cells in the field following Feulgen staining as described (Bacus *et al.*, 1990, Mol. Carcinog. 3:350-362), and the other for quantitating the level of HER2, HER3, and HER4 proteins following immunostaining. When the total DNA amount per cell was known, the average total HER2, HER3, and HER4 per cell were computed. Sparsely growing AU565 cells were used for calibrating the HER2 protein. Their level of staining was defined as 100% of HER2 protein content (1.0 relative amounts = 10,000 sum of optical density); all other measurements of HER2, HER3, and HER4 protein were related to this value.

14.1.10. Determination of the LD_{50} of HAR-TX β2

For toxicity studies, HAR-TX β2 at different concentrations was administered intravenous in 0.2 ml

PBS. Per group each two mice and two rats were injected.

14.2. RESULTS

14.2.1. Construction, Expression, and Purification of HAR-TX β2

The HAR-TX β2 expression plasmid, encoding the hydrophilic leader sequence from amphiregulin (AR), heregulin β2, and PE40, under control of the IPTG inducible T7 promoter, was constructed as described in Section 15.1.2., *supra*, and is diagrammatically shown in FIG. 23, Panel 1. The AR leader sequence was added to the N-terminus of heregulin to facilitate the purification procedure (FIG. 23, Panel 2). FIG. 24A and 24B show the nucleotide sequence and the deduced amino acid sequence of the cDNA encoding HAR-TX β2

Chimeric HAR-TX β2 protein was expressed in *E. coli* of inclusion bodies. Recombinant protein was denatured and refolded as described in Section 15.1.2., *supra*, and applied to cation-exchange chromatography on a POROS HS column. Semi-purified HAR-TX β2 protein was detected by PAGE and Coomassie blue staining as major band migrating at 51 kDa (FIG. 25, lane 2). The column flow-through from POROS HS contained only small amounts of HAR-TX β2 (FIG. 25, lane 3). POROS HS chromatography resulted in >50% purity of HAR-TX β2 (FIG. 25, lane 4). Further purification, to >95% purity, was done by chromatography using Source 15S cation-exchange resin (FIG. 25, lane 5). The monomeric nature of purified HAR-TX β2 was determined by non-reducing SDS-PAGE (FIG. 25, lane 6) which exhibited the same migration pattern as under reducing conditions (FIG. 25, lane 5).

14.2.2. Binding of HAR-TX β2 to MDA-MB-453 Cell Membranes

To determine the specific binding activity of HAR-TX β2, an ELISA assay was performed using membranes of the HER4 positive human breast carcinoma cell line MDA-MB-453 as the target for binding. HAR-TX β2 was found to bind to the immobilized cell membranes in a dose-dependent fashion up to 300 μg/ml (FIG. 26). PE40, the toxin component of HAR-TX β2 used as negative control, was unable to bind to MDA-MB-453 membranes.

14.2.3. Tyrosine Phosphorylation of HER Forms on Transfected CEM Cells

To test the biological activity of HAR-TX β2 a HER4 receptor phosphorylation assay was performed as previously described for heregulin (Carraway *et al.*, 1994, J. Biol. Chem. 269:14303-14306). CEM cells expressing different HER family members were exposed to HAR-TX β2 and stimulation of tyrosine phosphorylation was analyzed by phosphotyrosine immunoblot analysis (Section 4, *supra*; Section 15.1.5., *supra*). As shown in FIG. 27, HAR-TX β2 induced tyrosine phosphorylation in CEM cells expressing HER4 either alone or together with HER2, but not in cells expressing only HER2 or HER1. This result demonstrates that HER4 is sufficient and necessary for induction of tyrosine phosphorylation in response to HAR-TX β2, which is not true for HER1 and for HER2. The fact that HAR-TX β2 does not induce tyrosine phosphorylation in CEM cells transfected with HER1 confirms that the hydrophilic leader sequence of amphiregulin does not affect the specificity of the

heregulin moiety in its selective interaction between receptor family members.

14.2.4. Cytotoxicity of HAR-TX β2 Against Tumor Cells

The cell killing activity of HAR-TX β2 was determined against a variety of human cancer cell lines. AU565 and SKBR3 breast carcinomas and LNCaP prostate carcinoma were sensitive to HAR-TX β2 with EC_{50} values of 25, 20, 4.5 ng/ml, respectively, while SKOV3 ovarian carcinoma cells were insensitive to HAR-TX β2 (EC_{50} >2000 ng/ml) (FIG. 28, Panel 1). Addition of heregulin β2-Ig to LNCaP cells reduced the cytotoxic activity of HAR-TX β2 (FIG. 28, Panel 2). In contrast, L6-Ig, a chimeric mouse-human antibody with a non-related specificity but matching human Fc domains (Hellström *et al., supra*), did not inhibit the HAR-TX β2 cytotoxic activity (FIG. 28, Panel 2). Thus, the cytotoxic effect of HAR-TX β2 was due to specific heregulin-mediated binding. Similar data were obtained using MDA-MB-453 cells (not shown).

14.2.5. HER2, HER3, and HER4 Receptor Density on Human Tumor Cells: Correlation with HAR-TX β2-Mediated Cytotoxicity

To understand why cell lines differed in their sensitivity to HAR-TX β2, their levels of HER2, HER3, and HER4 were quantitated by image analysis (see Section 15.1.8. and 15.1.9., *supra*) using receptor specific monoclonal antibodies (Table IV). The data strongly indicate that HER4 expression is required for heregulin directed cytotoxic activity. All seven of the tumor cell lines which expressed detectable levels of HER4 were found to be sensitive to HAR-TX β2-

mediated killing with EC_{50} values ranging from 1-125 ng/ml. Moreover, the sensitivity of the different cell lines correlates directly with the expression level of HER4: MCF-7 cells displaying the lowest detectable levels of HER4 were found to be the least sensitive (EC_{50} = 125 ng/ml) of the cells which did respond. All four cell lines which were found to be devoid of any detectable HER4 expression on their surface were found to be resistant to HAR-TX β2. Three of them, SKOV3, L2987 and H3396, displayed both HER2 and HER3 in the absence of HER4.

TABLE IV

Comparative HER2, HER3, and HER4 cell surface receptor density and cytotoxicity of HAR-TX β2 on human tumor cell lines

		RELATIVE AMOUNTS			
Cell Line	Type	HER2	HER3	HER4	EC_{50}, (ng/ml)
BT474	Breast	1.6	0.32	0.3	1
MDA-MD-453	Breast	1.2	1.08	0.3	2
LNCaP	Prostate	0.7	2.6	0.85	4.5
T47D	Breast	0.04	0.1	0.1	9.5
SKBR3	Breast	4.6	2.5	0.56	20
AU565	Breast	4.6	0.73	0.18	25
MCF-7	Breast	0.04	1.8	0.05	125
H3396	Breast	0.6	2.5	--	>2000
SK0V3	Ovarian	0.64	1.3	--	>2000
L2987	Lung	0.16	1.4	--	>2000
CEM	T leukemia	--	--	--	>2000

14.2.6. HAR-TX β2 Induces Tyrosine Phosphorylation in Tumor Cells That Do Not Express HER4

In contrast to reports that heregulin directly binds to both HER3 and HER2/HER3 in a heterodimer configuration (Carraway *et al.*, 1994, J. Biol. Chem. 269:14303-14306; Sliwkowski *et al.*, 1994, J. Biol. Chem. 269:14661-15665), tumor cells that express HER3 alone (L2987) or co-express HER2 and HER3 (H3396 and SKOV3) were insensitive to HAR-TX β2. Direct interaction of H3396 and L2987 cells with the chimeric protein was determined by phosphotyrosine immunoblots following HAR-TX β2 induction. HAR-TX β2 was found to induce tyrosine phosphorylation in both tumor cell types (FIG. 29) similar to that previously seen in COS-7 cells transfected with HER2 and HER3 (Sliwkowski *et al., supra*). SKOV3 cells were found to exhibit the same tyrosine phosphorylation pattern in the presence or absence of heregulin and thus direct interaction between receptors and heregulin could not be established (data not shown). However, previous studies indicate that heregulin does not bind to these cells (Peles *et al., supra*).

14.2.7. Toxicity of HAT-TX β2

For the toxicity studies, HAR-TX β2 was administered as described in section 15.1.10. In mice, 2/2 animals died at 2 mg/kg, 2/2 died at 1 mg/kg, 1/2 died at 0.75 mg/kg, and 0/2 died at 0.5 mg/kg, thus the LD_{50} is about 0.75 mg/kg (Table V). In rats the determined LD_{50} was slightly higher, as 50% of the animals died at 1 mg/kg (Table V).

TABLE V
Toxicity of HAR-TX $\beta 2$

Species	dose[mg/ng]	Lethality [%]
mouse	0.5	0
	0.75	50
	1	100
	2	100
rat	1	50
	2	100

14.2.8. Characteristics of HER4 Specific Monoclonal Antibodies

The characteristics of the HER4 specific monoclonal antibodies disclosed herein are summarized in Table VI.

TABLE VI
Characteristics of HER4 Antibodies

Abbreviations: Cyto, cytoplasmic domain; ECD, extracellular domain; FACS, fluorescence-activated cell sorter analysis; fibro, fibroblasts; ICC, immunocytochemistry; RIP, receptor immunoprecipitation;

Hybridoma	Isotype	RIP	Western	Domain	FACS	HER4Ig + HER2Ig	ICC fibro.	ICC CHO/E4
6-4-11	IgG1	++	-	ECD	-	-	-	++++
7-142	IgG2a	-	++	Cyto	-	-	-	-

15. Microorganism and Cell Deposits

The following microorganisms and cell lines have been deposited with the American Type Culture Collection,

and have been assigned the following accession numbers:

Microorganism	Plasmid	Accession Number
E.coli SCS-1	pBSHER4Y	69131

(containing the complete human HER4 coding sequence)

Cell Line	Accession Number
CHO/HER4 21-2	CRL11205
Hybridoma Cell line 6-4-11	HB11715
Hybridoma Cell line 7-142	HB11716

The present invention is not to be limited in scope by the microorganisms and cell lines deposited or the embodiments disclosed herein, which are intended as single illustrations of one aspect of the invention, and any which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims. All base pair and amino acid residue numbers and sizes given for polynucleotides and polypeptides are approximate and used for the purpose of description.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which the invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: HER4 HUMAN RECEPTOR TYROSINE KINASE

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 34..3961

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AATTGTCAGC ACGGGATCTG AGACTTCCAA AAA ATG AAG CCG GCG ACA GGA CTT      54
                                      Met Lys Pro Ala Thr Gly Leu
                                        1               5

TGG GTC TGG GTG AGC CTT CTC GTG GCG GCG GGG ACC GTC CAG CCC AGC     102
Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
          10                  15                  20

GAT TCT CAG TCA GTG TGT GCA GGA ACG GAG AAT AAA CTG AGC TCT CTC     150
Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
      25                  30                  35

TCT GAC CTG GAA CAG CAG TAC CGA GCC TTG CGC AAG TAC TAT GAA AAC     198
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn
  40                  45                  50                  55

TGT GAG GTT GTC ATG GGC AAC CTG GAG ATA ACC AGC ATT GAG CAC AAC     246
Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn
                  60                  65                  70

CGG GAC CTC TCC TTC CTG CGG TCT GTT CGA GAA GTC ACA GGC TAC GTG     294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val
              75                  80                  85

TTA GTG GCT CTT AAT CAG TTT CGT TAC CTG CCT CTG GAG AAT TTA CGC     342
Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg
          90                  95                  100

ATT ATT CGT GGG ACA AAA CTT TAT GAG GAT CGA TAT GCC TTG GCA ATA     390
Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile
      105                 110                 115

TTT TTA AAC TAC AGA AAA GAT GGA AAC TTT GGA CTT CAA GAA CTT GGA     438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly
120                 125                 130                 135

TTA AAG AAC TTG ACA GAA ATC CTA AAT GGT GGA GTC TAT GTA GAC CAG     486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln
      140                 145                 150

AAC AAA TTC CTT TGT TAT GCA GAC ACC ATT CAT TGG CAA GAT ATT GTT     534
Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val
              155                 160                 165

CGG AAC CCA TGG CCT TCC AAC TTG ACT CTT GTG TCA ACA AAT GGT AGT     582
Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser
170                 175                 180                 185

TCA GGA TGT GGA CGT TGC CAT AAG TCC TGT ACT GGC CGT TGC TGG GGA     630
Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly
                  190                 195

CCC ACA GAA AAT CAT TGC CAG ACT TTG ACA AGG ACG GTG TGT GCA GAA     678
Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu
200                 205                 210                 215

CAA TGT GAC GGC AGA TGC TAC GGA CCT TAC GTC AGT GAC TGC TGC CAT     726
Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His
                  220                 225                 230

CGA GAA TGT GCT GGA GGC TGC TCA GGA CCT AAG GAC ACA GAC TGC TTT     774
Arg-Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe
              235                 240                 245
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GCC TGC ATG AAT TTC AAT GAC AGT GGA GCA TGT GTT ACT CAG TGT CCC      822
Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro
        250                 255                 260

CAA ACC TTT GTC TAC AAT CCA ACC ACC TTT CAA CTG GAG CAC AAT TTC      870
Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe
    265                 270                 275

AAT GCA AAG TAC ACA TAT GGA GCA TTC TGT GTC AAG AAA TGT CCA CAT      918
Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His
280                 285                 290                 295

AAC TTT GTG GTA GAT TCC AGT TCT TGT GTG CGT GCC TGC CCT AGT TCC      966
Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser
                300                 305                 310

AAG ATG GAA GTA GAA GAA AAT GGG ATT AAA ATG TGT AAA CCT TGC ACT     1014
Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr
            315                 320                 325

GAC ATT TGC CCA AAA GCT TGT GAT GGC ATT GGC ACA GGA TCA TTG ATG     1062
Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met
        330                 335                 340

TCA GCT CAG ACT GTG GAT TCC AGT AAC ATT GAC AAA TTC ATA AAC TGT     1110
Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys
    345                 350                 355

ACC AAG ATC AAT GGG AAT TTG ATC TTT CTA GTC ACT GGT ATT CAT GGG     1158
Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly
360                 365                 370                 375

GAC CCT TAC AAT GCA ATT GAA GCC ATA GAC CCA GAG AAA CTG AAC GTC     1206
Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val
                380                 385                 390

TTT CGG ACA GTC AGA GAG ATA ACA GGT TTC CTG AAC ATA CAG TCA TGG     1254
Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp
            395                 400                 405

CCA CCA AAC ATG ACT GAC TTC AGT GTT TTT TCT AAC CTG GTG ACC ATT     1302
Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile
        410                 415                 420

GGT GGA AGA GTA CTC TAT AGT GGC CTG TCC TTG CTT ATC CTC AAG CAA     1350
Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln
    425                 430                 435

CAG GGC ATC ACC TCT CTA CAG TTC CAG TCC CTG AAG GAA ATC AGC GCA     1398
Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala
440                 445                 450                 455

GGA AAC ATC TAT ATT ACT GAC AAC AGC AAC CTG TGT TAT TAT CAT ACC     1446
Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr
                460                 465                 470

ATT AAC TGG ACA ACA CTC TTC AGC ACA ATC AAC CAG AGA ATA GTA ATC     1494
Ile Asn Trp Thr Thr Leu Phe Ser Thr Ile Asn Gln Arg Ile Val Ile
            475                 480                 485

CGG GAC AAC AGA AAA GCT GAA AAT TGT ACT GCT GAA GGA ATG GTG TGC     1542
Arg Asp Asn Arg Lys Ala Glu Asn Cys Thr Ala Glu Gly Met Val Cys
        490                 495                 500

AAC-CAT CTG TGT TCC AGT GAT GGC TGT TGG GGA CCT GGG CCA GAC CAA     1590
Asn His Leu Cys Ser Ser Asp Gly Cys Trp Gly Pro Gly Pro Asp Gln
    505                 510                 515
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TGT CTG TCG TGT CGC CGC TTC AGT AGA GGA AGG ATC TGC ATA GAG TCT      1638
Cys Leu Ser Cys Arg Arg Phe Ser Arg Gly Arg Ile Cys Ile Glu Ser
520                 525                 530                 535

TGT AAC CTC TAT GAT GGT GAA TTT CGG GAG TTT GAG AAT GGC TCC ATC      1686
Cys Asn Leu Tyr Asp Gly Glu Phe Arg Glu Phe Glu Asn Gly Ser Ile
                540                 545                 550

TGT GTG GAG TGT GAC CCC CAG TGT GAG AAG ATG GAA GAT GGC CTC CTC      1734
Cys Val Glu Cys Asp Pro Gln Cys Glu Lys Met Glu Asp Gly Leu Leu
            555                 560                 565

ACA TGC CAT GGA CCG GGT CCT GAC AAC TGT ACA AAG TGC TCT CAT TTT      1782
Thr Cys His Gly Pro Gly Pro Asp Asn Cys Thr Lys Cys Ser His Phe
        570                 575                 580

AAA GAT GGC CCA AAC TGT GTG GAA AAA TGT CCA GAT GGC TTA CAG GGG      1830
Lys Asp Gly Pro Asn Cys Val Glu Lys Cys Pro Asp Gly Leu Gln Gly
    585                 590                 595

GCA AAC AGT TTC ATT TTC AAG TAT GCT GAT CCA GAT CGG GAG TGC CAC      1878
Ala Asn Ser Phe Ile Phe Lys Tyr Ala Asp Pro Asp Arg Glu Cys His
600                 605                 610                 615

CCA TGC CAT CCA AAC TGC ACC CAA GGG TGT AAC GGT CCC ACT AGT CAT      1926
Pro Cys His Pro Asn Cys Thr Gln Gly Cys Asn Gly Pro Thr Ser His
                620                 625                 630

GAC TGC ATT TAC TAC CCA TGG ACG GGC CAT TCC ACT TTA CCA CAA CAT      1974
Asp Cys Ile Tyr Tyr Pro Trp Thr Gly His Ser Thr Leu Pro Gln His
            635                 640                 645

GCT AGA ACT CCC CTG ATT GCA GCT GGA GTA ATT GGT GGG CTC TTC ATT      2022
Ala Arg Thr Pro Leu Ile Ala Ala Gly Val Ile Gly Gly Leu Phe Ile
        650                 655                 660

CTG GTC ATT GTG GGT CTG ACA TTT GCT GTT TAT GTT AGA AGG AAG AGC      2070
Leu Val Ile Val Gly Leu Thr Phe Ala Val Tyr Val Arg Arg Lys Ser
    665                 670                 675

ATC AAA AAG AAA AGA GCC TTG AGA AGA TTC TTG GAA ACA GAG TTG GTG      2118
Ile Lys Lys Lys Arg Ala Leu Arg Arg Phe Leu Glu Thr Glu Leu Val
680                 685                 690                 695

GAA CCA TTA ACT CCC AGT GGC ACA GCA CCC AAT CAA GCT CAA CTT CGT      2166
Glu Pro Leu Thr Pro Ser Gly Thr Ala Pro Asn Gln Ala Gln Leu Arg
                700                 705                 710

ATT TTG AAA GAA ACT GAG CTG AAG AGG GTA AAA GTC CTT GGC TCA GGT      2214
Ile Leu Lys Glu Thr Glu Leu Lys Arg Val Lys Val Leu Gly Ser Gly
            715                 720                 725

GCT TTT GGA ACG GTT TAT AAA GGT ATT TGG GTA CCT GAA GGA GAA ACT      2262
Ala Phe Gly Thr Val Tyr Lys Gly Ile Trp Val Pro Glu Gly Glu Thr
        730                 735                 740

GTG AAG ATT CCT GTG GCT ATT AAG ATT CTT AAT GAG ACA ACT GGT CCC      2310
Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro
    745                 750                 755

AAG GCA AAT GTG GAG TTC ATG GAT GAA GCT CTG ATC ATG GCA AGT ATG      2358
Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met
760                 765                 770                 775

GAT CAT CCA CAC CTA GTC CGG TTG CTG GGT GTG TGT CTG AGC CCA ACC      2406
Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr
                780                 785                 790
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ATC CAG CTG GTT ACT CAA CTT ATG CCC CAT GGC TGC CTG TTG GAG TAT      2454
Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr
            795                 800                 805

GTC CAC GAG CAC AAG GAT AAC ATT GGA TCA CAA CTG CTG CTT AAC TGG      2502
Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp
        810                 815                 820

TGT GTC CAG ATA GCT AAG GGA ATG ATG TAC CTG GAA GAA AGA CGA CTC      2550
Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu
    825                 830                 835

GTT CAT CGG GAT TTG GCA GCC CGT AAT GTC TTA GTG AAA TCT CCA AAC      2598
Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn
840                 845                 850                 855

CAT GTG AAA ATC ACA GAT TTT GGG CTA GCC AGA CTC TTG GAA GGA GAT      2646
His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp
                860                 865                 870

GAA AAA GAG TAC AAT GCT GAT GGA GGA AAG ATG CCA ATT AAA TGG ATG      2694
Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met
            875                 880                 885

GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT GAC GTT      2742
Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val
        890                 895                 900

TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA GGA AAA      2790
Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys
    905                 910                 915

CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA GAG AAA      2838
Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys
920                 925                 930                 935

GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT TAC ATG      2886
Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met
                940                 945                 950

GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT AAA TTT      2934
Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe
            955                 960                 965

AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT CAA AGA      2982
Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg
        970                 975                 980

TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT CCA AAT      3030
Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn
    985                 990                 995

GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG GAA GAT      3078
Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp
1000                1005                1010                1015

ATG ATG GAT GCT GAG GAG TAC TTG GTC CCT CAG GCT TTC AAC ATC CCA      3126
Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro
    1020                1025                1030

CCT CCC ATC TAT ACT TCC AGA GCA AGA ATT GAC TCG AAT AGG AGT GAA      3174
Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Glu
1035                1040                1045

ATT GGA CAC AGC CCT CCT CCT GCC TAC ACC CCC ATG TCA GGA AAC CAG      3222
Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly Asn Gln
                1055                1060
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TTT GTA TAC CGA GAT GGA GGT TTT GCT GCT GAA CAA GGA GTG TCT GTG      3270
Phe Val Tyr Arg Asp Gly Gly Phe Ala Ala Glu Gln Gly Val Ser Val
            1070                1075

CCC TAC AGA GCC CCA ACT AGC ACA ATT CCA GAA GCT CCT GTG GCA CAG      3318
Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val Ala Gln
1080            1085                1090                1095

GGT GCT ACT GCT GAG ATT TTT GAT GAC TCC TGC TGT AAT GGC ACC CTA      3366
Gly Ala Thr Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly Thr Leu
    1100                1105                1110

CGC AAG CCA GTG GCA CCC CAT GTC CAA GAG GAC AGT AGC ACC CAG AGG      3414
Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr Gln Arg
1115                1120                1125

TAC AGT GCT GAC CCC ACC GTG TTT GCC CCA GAA CGG AGC CCA CGA GGA      3462
Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro Arg Gly
                1135                1140

GAG CTG GAT GAG GAA GGT TAC ATG ACT CCT ATG CGA GAC AAA CCC AAA      3510
Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys Pro Lys
            1150                1155

CAA GAA TAC CTG AAT CCA GTG GAG GAG AAC CCT TTT GTT TCT CGG AGA      3558
Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser Arg Arg
        1165                1170                1175

AAA AAT GGA GAC CTT CAA GCA TTG GAT AAT CCC GAA TAT CAC AAT GCA      3606
Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His Asn Ala
    1180                1185                1190

TCC AAT GGT CCA CCC AAG GCC GAG GAT GAG TAT GTG AAT GAG CCA CTG      3654
Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu Pro Leu
1195                1200                1205

TAC CTC AAC ACC TTT GCC AAC ACC TTG GGA AAA GCT GAG TAC CTG AAG      3702
Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr Leu Lys
                1215                1220

AAC AAC ATA CTG TCA ATG CCA GAG AAG GCC AAG AAA GCG TTT GAC AAC      3750
Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe Asp Asn
            1230                1235

CCT GAC TAC TGG AAC CAC AGC CTG CCA CCT CGG AGC ACC CTT CAG CAC      3798
Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu Gln His
        1245                1250                1255

CCA GAC TAC CTG CAG GAG TAC AGC ACA AAA TAT TTT TAT AAA CAG AAT      3846
Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys Gln Asn
    1260                1265                1270

GGG CGG ATC CGG CCT ATT GTG GCA GAG AAT CCT GAA TAC CTC TCT GAG      3894
Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu Ser Glu
1275                1280                1285

TTC TCC CTG AAG CCA GGC ACT GTG CTG CCG CCT CCA CCT TAC AGA CAC      3942
Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr Arg His
                1295                1300

CGG AAT ACT GTG GTG TAAGCTCAGT TGTGGTTTTT TAGGTGGAGA GACACACCTG      3997
Arg Asn Thr Val Val

CTGCAATTTC CCCACCCCCC TCTCTTTCTC TGGTGGTCTT CCTTCTACCC CAAGGCCAGT    4057

AGTTTTGACA CTTCCCAGTG GAAGATACAG AGATGCAATG ATAGTTATGT GCTTACCTAA    4117
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CTTGAACATT AGAGGGAAAG ACTGAAAGAG AAAGATAGGA GGAACCACAA TGTTTCTTCA 4177

TTTCTCTGCA TGGGTTGGTC AGGAGAATGA AACAGCTAGA GAAGGACCAG AAAATGTAAG 4237

GCAATGCTGC CTACTATCAA ACTAGCTGTC ACTTTTTTTC TTTTTCTTTT TCTTTCTTTG 4297

TTTCTTTCTT CCTCTTCTTT TTTTTTTTTT TTTTAAAGCA GATGGTTGAA ACACCCATGC 4357

TATCTGTTCC TATCTGCAGG AACTGATGTG TGCATATTTA GCATCCCTGG AAATCATAAT 4417

AAAGTTTCCA TTAGAACAAA AGAATAACAT TTTCTATAAC ATATGATAGT GTCTGAAATT 4477

GAGAATCCAG TTTCTTTCCC CAGCAGTTTC TGTCCTAGCA AGTAAGAATG GCCAACTCAA 4537

CTTTCATAAT TTAAAAATCT CCATTAAAGT TATAACTAGT AATTATGTTT TCAACACTTT 4597

TTGGTTTTTT TCATTTTGTT TTGCTCTGAC CGATTCCTTT ATATTTGCTC CCCTATTTTT 4657

GGCTTTAATT TCTAATTGCA AAGATGTTTA CATCAAAGCT TCTTCACAGA ATTTAAGCAA 4717

GAAATATTTT AATATAGTGA AATGGCCACT ACTTTAAGTA TACAATCTTT AAAATAAGAA 4777

AGGGAGGCTA ATATTTTTCA TGCTATCAAA TTATCTTCAC CCTCATCCTT TACATTTTTC 4837

AACATTTTTT TTTCTCCATA AATGACACTA CTTGATAGGC CGTTGGTTGT CTGAAGAGTA 4897

GAAGGGAAAC TAAGAGACAG TTCTCTGTGG TTCAGGAAAA CTACTGATAC TTTCAGGGGT 4957

GGCCCAATGA GGGAATCCAT TGAACTGGAA GAAACACACT GGATTGGGTA TGTCTACCTG 5017

GCAGATACTC AGAAATGTAG TTTGCACTTA AGCTGTAATT TTATTTGTTC TTTTTCTGAA 5077

CTCCATTTTG GATTTTGAAT CAAGCAATAT GGAAGCAACC AGCAAATTAA CTAATTTAAG 5137

TACATTTTTA AAAAAAGAGC TAAGATAAAG ACTGTGGAAA TGCCAAACCA AGCAAATTAG 5197

GAACCTTGCA ACGGTATCCA GGGACTATGA TGAGAGGCCA GCACATTATC TTCATATGTC 5257

ACCTTTGCTA CGCAAGGAAA TTTGTTCAGT TCGTATACTT CGTAAGAAGG AATGCGAGTA 5317

AGGATTGGCT TGAATTCCAT GGAATTTCTA GTATGAGACT ATTTATATGA AGTAGAAGGT 5377

AACTCTTTGC ACATAAATTG GTATAATAAA AAGAAAAACA CAAACATTCA AAGCTTAGGG 5437

ATAGGTCCTT GGGTCAAAAG TTGTAAATAA ATGTGAAACA TCTTCTCAAA AAAAAAAAAA 5497

AAAA 5501

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1308 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
1 5 10 15

Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
20 25 30

Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala

35 40 45

Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
50 55 60

Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
65 70 75 80

Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
85 90 95

Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
100 105 110

Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
115 120 125

Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
130 135 140

Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
145 150 155 160

Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
165 170 175

Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
180 185 190

Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
195 200 205

Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
210 215 220

Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
225 230 235 240

Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
245 250 255

Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
260 265 270

Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
275 280 285

Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
290 295 300

Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
305 310 315 320

Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
325 330 335

Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
340 345 350

Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
355 360 365

Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
370 375 380

Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
385 390 395 400

Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
405 410 415

Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
420 425 430

Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
435 440 445

Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
450 455 460

Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
465 470 475 480

Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
485 490 495

Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
500 505 510

Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
515 520 525

Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
530 535 540

Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
545 550 555 560

Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
565 570 575

Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
580 585 590

Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
595 600 605

Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
610 615 620

Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
625 630 635 640

His Ser Thr Leu Pro Gln His Ala Arg Thr Pro Leu Ile Ala Ala Gly
645 650 655

Val Ile Gly Gly Leu Phe Ile Leu Val Ile Val Gly Leu Thr Phe Ala
660 665 670

Val Tyr Val Arg Arg Lys Ser Ile Lys Lys Lys Arg Ala Leu Arg Arg
675 680 685

Phe Leu Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Thr Ala
690 695 700

Pro Asn Gln Ala Gln Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg
705 710 715 720

Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile
725 730 735

Trp Val Pro Glu Gly Glu Thr Val Lys Ile Pro Val Ala Ile Lys Ile
740 745 750

Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu

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        755                 760                 765

Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu
    770                 775                 780

Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro
785                 790                 795                 800

His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly
                805                 810                 815

Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met
            820                 825                 830

Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn
        835                 840                 845

Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu
    850                 855                 860

Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly
865                 870                 875                 880

Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys
                885                 890                 895

Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu
            900                 905                 910

Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu
        915                 920                 925

Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
    930                 935                 940

Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
945                 950                 955                 960

Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
                965                 970                 975

Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
            980                 985                 990

Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
        995                 1000                1005

Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
    1010                1015                1020

Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
1025                1030                1035                1040

Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr
                1045                1050                1055

Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe Ala
            1060                1065                1070

Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile
        1075                1080                1085

Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp Asp
    1090                1095                1100

Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val Gln
1105                1110                1115                1120
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Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe Ala
1125 1130 1135

Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met Thr
1140 1145 1150

Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu Glu
1155 1160 1165

Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu Asp
1170 1175 1180

Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu Asp
1185 1190 1195 1200

Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr Leu
1205 1210 1215

Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu Lys
1220 1225 1230

Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro
1235 1240 1245

Pro Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr
1250 1255 1260

Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu
1265 1270 1275 1280

Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu
1285 1290 1295

Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
1300 1305

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5555 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 34..3210

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTGTCAGC ACGGGATCTG AGACTTCCAA AAA ATG AAG CCG GCG ACA GGA CTT 54
Met Lys Pro Ala Thr Gly Leu
1 5

TGG GTC TGG GTG AGC CTT CTC GTG GCG GCG GGG ACC GTC CAG CCC AGC 102
Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
15 20

GAT TCT CAG TCA GTG TGT GCA GGA ACG GAG AAT AAA CTG AGC TCT CTC 150
Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
30 35

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TCT GAC CTG GAA CAG CAG TAC CGA GCC TTG CGC AAG TAC TAT GAA AAC      198
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn
          45                  50                  55

TGT GAG GTT GTC ATG GGC AAC CTG GAG ATA ACC AGC ATT GAG CAC AAC      246
Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn
      60                  65                  70

CGG GAC CTC TCC TTC CTG CGG TCT GTT CGA GAA GTC ACA GGC TAC GTG      294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val
 75                  80                  85

TTA GTG GCT CTT AAT CAG TTT CGT TAC CTG CCT CTG GAG AAT TTA CGC      342
Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg
                95                 100

ATT ATT CGT GGG ACA AAA CTT TAT GAG GAT CGA TAT GCC TTG GCA ATA      390
Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile
            110                 115

TTT TTA AAC TAC AGA AAA GAT GGA AAC TTT GGA CTT CAA GAA CTT GGA      438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly
        125                 130                 135

TTA AAG AAC TTG ACA GAA ATC CTA AAT GGT GGA GTC TAT GTA GAC CAG      486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln
    140                 145                 150

AAC AAA TTC CTT TGT TAT GCA GAC ACC ATT CAT TGG CAA GAT ATT GTT      534
Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val
155                 160                 165

CGG AAC CCA TGG CCT TCC AAC TTG ACT CTT GTG TCA ACA AAT GGT AGT      582
Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser
                175                 180

TCA GGA TGT GGA CGT TGC CAT AAG TCC TGT ACT GGC CGT TGC TGG GGA      630
Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly
            190                 195

CCC ACA GAA AAT CAT TGC CAG ACT TTG ACA AGG ACG GTG TGT GCA GAA      678
Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu
        205                 210                 215

CAA TGT GAC GGC AGA TGC TAC GGA CCT TAC GTC AGT GAC TGC TGC CAT      726
Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His
    220                 225                 230

CGA GAA TGT GCT GGA GGC TGC TCA GGA CCT AAG GAC ACA GAC TGC TTT      774
Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe
235                 240                 245

GCC TGC ATG AAT TTC AAT GAC AGT GGA GCA TGT GTT ACT CAG TGT CCC      822
Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro
                255                 260

CAA ACC TTT GTC TAC AAT CCA ACC ACC TTT CAA CTG GAG CAC AAT TTC      870
Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe
            270                 275

AAT GCA AAG TAC ACA TAT GGA GCA TTC TGT GTC AAG AAA TGT CCA CAT      918
Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His
        285                 290                 295

AAC TTT GTG GTA GAT TCC AGT TCT TGT GTG CGT GCC TGC CCT AGT TCC      966
Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser
    300                 305                 310
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AAG ATG GAA GTA GAA GAA AAT GGG ATT AAA ATG TGT AAA CCT TGC ACT      1014
Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr
315                 320                 325

GAC ATT TGC CCA AAA GCT TGT GAT GGC ATT GGC ACA GGA TCA TTG ATG      1062
Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met
            335                 340

TCA GCT CAG ACT GTG GAT TCC AGT AAC ATT GAC AAA TTC ATA AAC TGT      1110
Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys
        350                 355

ACC AAG ATC AAT GGG AAT TTG ATC TTT CTA GTC ACT GGT ATT CAT GGG      1158
Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly
    365                 370                 375

GAC CCT TAC AAT GCA ATT GAA GCC ATA GAC CCA GAG AAA CTG AAC GTC      1206
Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val
380                 385                 390

TTT CGG ACA GTC AGA GAG ATA ACA GGT TTC CTG AAC ATA CAG TCA TGG      1254
Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp
395                 400                 405

CCA CCA AAC ATG ACT GAC TTC AGT GTT TTT TCT AAC CTG GTG ACC ATT      1302
Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile
            415                 420

GGT GGA AGA GTA CTC TAT AGT GGC CTG TCC TTG CTT ATC CTC AAG CAA      1350
Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln
        430                 435

CAG GGC ATC ACC TCT CTA CAG TTC CAG TCC CTG AAG GAA ATC AGC GCA      1398
Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala
    445                 450                 455

GGA AAC ATC TAT ATT ACT GAC AAC AGC AAC CTG TGT TAT TAT CAT ACC      1446
Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr
460                 465                 470

ATT AAC TGG ACA ACA CTC TTC AGC ACA ATC AAC CAG AGA ATA GTA ATC      1494
Ile Asn Trp Thr Thr Leu Phe Ser Thr Ile Asn Gln Arg Ile Val Ile
475                 480                 485

CGG GAC AAC AGA AAA GCT GAA AAT TGT ACT GCT GAA GGA ATG GTG TGC      1542
Arg Asp Asn Arg Lys Ala Glu Asn Cys Thr Ala Glu Gly Met Val Cys
            495                 500

AAC CAT CTG TGT TCC AGT GAT GGC TGT TGG GGA CCT GGG CCA GAC CAA      1590
Asn His Leu Cys Ser Ser Asp Gly Cys Trp Gly Pro Gly Pro Asp Gln
        510                 515

TGT CTG TCG TGT CGC CGC TTC AGT AGA GGA AGG ATC TGC ATA GAG TCT      1638
Cys Leu Ser Cys Arg Arg Phe Ser Arg Gly Arg Ile Cys Ile Glu Ser
    525                 530                 535

TGT AAC CTC TAT GAT GGT GAA TTT CGG GAG TTT GAG AAT GGC TCC ATC      1686
Cys Asn Leu Tyr Asp Gly Glu Phe Arg Glu Phe Glu Asn Gly Ser Ile
540                 545                 550

TGT GTG GAG TGT GAC CCC CAG TGT GAG AAG ATG GAA GAT GGC CTC CTC      1734
Cys Val Glu Cys Asp Pro Gln Cys Glu Lys Met Glu Asp Gly Leu Leu
555                 560                 565

ACA TGC CAT GGA CCG GGT CCT GAC AAC TGT ACA AAG TGC TCT CAT TTT      1782
Thr Cys His Gly Pro Gly Pro Asp Asn Cys Thr Lys Cys Ser His Phe
            575                 580
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AAA GAT GGC CCA AAC TGT GTG GAA AAA TGT CCA GAT GGC TTA CAG GGG      1830
Lys Asp Gly Pro Asn Cys Val Glu Lys Cys Pro Asp Gly Leu Gln Gly
            590                 595

GCA AAC AGT TTC ATT TTC AAG TAT GCT GAT CCA GAT CGG GAG TGC CAC      1878
Ala Asn Ser Phe Ile Phe Lys Tyr Ala Asp Pro Asp Arg Glu Cys His
        605                 610                 615

CCA TGC CAT CCA AAC TGC ACC CAA GGG TGT AAC GGT CCC ACT AGT CAT      1926
Pro Cys His Pro Asn Cys Thr Gln Gly Cys Asn Gly Pro Thr Ser His
    620                 625                 630

GAC TGC ATT TAC TAC CCA TGG ACG GGC CAT TCC ACT TTA CCA CAA CAT      1974
Asp Cys Ile Tyr Tyr Pro Trp Thr Gly His Ser Thr Leu Pro Gln His
635                 640                 645

GCT AGA ACT CCC CTG ATT GCA GCT GGA GTA ATT GGT GGG CTC TTC ATT      2022
Ala Arg Thr Pro Leu Ile Ala Ala Gly Val Ile Gly Gly Leu Phe Ile
                655                 660

CTG GTC ATT GTG GGT CTG ACA TTT GCT GTT TAT GTT AGA AGG AAG AGC      2070
Leu Val Ile Val Gly Leu Thr Phe Ala Val Tyr Val Arg Arg Lys Ser
            670                 675

ATC AAA AAG AAA AGA GCC TTG AGA AGA TTC TTG GAA ACA GAG TTG GTG      2118
Ile Lys Lys Lys Arg Ala Leu Arg Arg Phe Leu Glu Thr Glu Leu Val
        685                 690                 695

GAA CCA TTA ACT CCC AGT GGC ACA GCA CCC AAT CAA GCT CAA CTT CGT      2166
Glu Pro Leu Thr Pro Ser Gly Thr Ala Pro Asn Gln Ala Gln Leu Arg
    700                 705                 710

ATT TTG AAA GAA ACT GAG CTG AAG AGG GTA AAA GTC CTT GGC TCA GGT      2214
Ile Leu Lys Glu Thr Glu Leu Lys Arg Val Lys Val Leu Gly Ser Gly
715                 720                 725

GCT TTT GGA ACG GTT TAT AAA GGT ATT TGG GTA CCT GAA GGA GAA ACT      2262
Ala Phe Gly Thr Val Tyr Lys Gly Ile Trp Val Pro Glu Gly Glu Thr
                735                 740

GTG AAG ATT CCT GTG GCT ATT AAG ATT CTT AAT GAG ACA ACT GGT CCC      2310
Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro
            750                 755

AAG GCA AAT GTG GAG TTC ATG GAT GAA GCT CTG ATC ATG GCA AGT ATG      2358
Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met
        765                 770                 775

GAT CAT CCA CAC CTA GTC CGG TTG CTG GGT GTG TGT CTG AGC CCA ACC      2406
Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr
    780                 785                 790

ATC CAG CTG GTT ACT CAA CTT ATG CCC CAT GGC TGC CTG TTG GAG TAT      2454
Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr
795                 800                 805

GTC CAC GAG CAC AAG GAT AAC ATT GGA TCA CAA CTG CTG CTT AAC TGG      2502
Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp
                815                 820

TGT GTC CAG ATA GCT AAG GGA ATG ATG TAC CTG GAA GAA AGA CGA CTC      2550
Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu
            830                 835

GTT CAT CGG GAT TTG GCA GCC CGT AAT GTC TTA GTG AAA TCT CCA AAC      2598
Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn
        845                 850                 855
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CAT GTG AAA ATC ACA GAT TTT GGG CTA GCC AGA CTC TTG GAA GGA GAT      2646
His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp
                        865                 870

GAA AAA GAG TAC AAT GCT GAT GGA GGA AAG ATG CCA ATT AAA TGG ATG      2694
Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met
875                 880                 885

GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT GAC GTT      2742
Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val
                895                 900

TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA GGA AAA      2790
Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys
            910                 915

CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA GAG AAA      2838
Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys
        925                 930                 935

GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT TAC ATG      2886
Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met
    940                 945                 950

GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT AAA TTT      2934
Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe
955                 960                 965

AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT CAA AGA      2982
Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg
                975                 980

TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT CCA AAT      3030
Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn
            990                 995

GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG GAA GAT      3078
Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp
        1005                1010                1015

ATG ATG GAT GCT GAG GAG TAC TTG GTC CCT CAG GCT TTC AAC ATC CCA      3126
Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro
    1020                1025                1030

CCT CCC ATC TAT ACT TCC AGA GCA AGA ATT GAC TCG AAT AGG AGT GTA      3174
Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Val
1035                1040                1045

AGA AAT AAT TAT ATA CAC ATA TCA TAT TCT TTC TGAGATATAA AATCATGTAA    3227
Arg Asn Asn Tyr Ile His Ile Ser Tyr Ser Phe
                1055

TAGTTCATAA GCACTAACAT TTCAAAATAA TTATATAGCT CAAATCAATG TGATGCCTAG    3287

ATTAAAAATA TACCATACCC ACAAAAGATG TGCCAATCTT GCTATATGTA GTTAATTTTG    3347

GAAGACAAGC ATGGACAATA CAACATGTAC TCTGAAATAC CTTCAAGATT TCAGAAGCAA    3407

AACATTTTCC TCATCTTAAT TTATTTAAAA CAAATCTTAA CTTTAAAAAA CAATTCCAAC    3467

TAATAAAACC ATTATGTGTA TATAAATAAA TGAAAATTCC TACCAAGTAG GCTTTCTACT    3527

TTTCTTTCTT AAAAAGATAT TATGATATAT TAGTCAAGAA GTAATACAAG TATAAATCTC    3587

TTTCACTTAT TTAAGAAAAA TTAAATATTT TCTGTCAAGT TGAAGTAGAA ACACAGAAAA    3647

CCGTGCAGTC CTTTGAACCT AATCACATCG AAAAGGCTGC TGAGAAGTAG ATTTTTGTTT    3707
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TTAAGAAGTA GATTTAAGTT TTGAAGGAAG TTTCTGAAAA CACTTTACAT TTTAAATGTT 3767

AAACCTACTC TATATGAATT CCATTCTTTC TTTGAAAGCT GTCAAATCCA TGCATTTATT 3827

TTTATAAATT CATTCCTCAT ACATTCAACA TATATTGAGT ACCACTGTAT GTGAAGCATT 3887

AGTATACATT TAAGACTCAA AGAATTTTGA TACAACTTCT GCTTTCAAGA AGTGAAAACC 3947

TTAATCAAAG AATCATACAG ATAGAGGGAC TGCATAGTAA GTGCTGTAAT CCAGTATTCA 4007

CTGACCAGTA CGGAGCATGA AGAAGTAGTA AATTTGTGTC TGTAATCAGT TTCTTCCATT 4067

GATAAGATAT AAACATGATG CTTAATTTTT TCTAGAAGAT AATTCTTTTC TCTTAATCTA 4127

AGAACATTAT CATAGCTAGT AGAACCGACA GCATCCGATT TCTCTTGACC ATAGCCATAA 4187

GAATATCTTC AACTTGCTGC TCATTATCTA ACAAACATAA TTTTCTTTAT TTCATATTGA 4247

TTGTAATAAG TAATATCCCC CTGGAAGTTT ACTATTCAAC ACATATATGT TAACCTCCTT 4307

AATTCCTTAA ACAAACTTCA TGAGGTTCTA TTATTATCAT CCCCTTCTTT CAAAGGAAGA 4367

AACTTGCCAC AGAGAAGTCA GGTGATATGA CTGGTGTCAC ACAGCTAGTC AGTGGAAGAG 4427

AGGAATAAGT AATCTAGATA TCTGCCTACT ACACTGTAGG TTTGCTTCAA AGTTACTGAA 4487

GYCATGTTAT TTCCATGATG TGATTAGAGT CTGGGACTTG TCTTGTTTGG GAAATTTCCC 4547

AGGTGGTTTT CTTATAAAAT GCATCTCAAA TCTGCTCTAC ACCTTTTACT CATCTACCTC 4607

CATTTAGAAG ATCTGATATG GAAAGAGACA AAGATGGAGA CCTCAATTAT TTTTTCTTTT 4667

CTGTTAAAAA TATTATAGTA CAACTGAAAC TTATCACATG CCAATGGGGA ATAGATAACT 4727

AAAAGTTTAA AATTAGATCA ATGGATAGGT AAATGAATAA TCNTTCTTTT GCTTGTGAGA 4787

GGGGAAGGAA AAGCGGTTAA GGTGGTATAA AGGAGGCTCC TCTGTACACT TGCAAAATGA 4847

TCAAATTATA TACCCTTGTA TTTATAATTT TAAGTGACAA ATTCATTACT TCTGGTTACA 4907

ACAGTGAAAT TTAAAAAAAA ATAGTTTTTC TTTCTTAGCT TGCAATGCTA TAAATCTTTT 4967

TCTTTTTATA AGAATTCTTA CATTTCAGCT TTTTGTTCAT TTTAATTTAT AATTCTCAGT 5027

GCAAGAAATT CTTAATAAAG GTTTGAGCTA GCTAGATGGA ATTATTGAGA CAAAGTCTAA 5087

ATCACCCGTG GACTTATTTG ACCTTTAGCC ATCATTTCTT ATTCCACATT ATAAAACAAT 5147

GTTACCTGTA GATTTCTTTT TACTTTTTCA GTCCTTGGAA AAGAAATGGT GATTAAATAT 5207

CATTATATCA TTTTATGTTC AGGCATTTAA AAAGCTTTAT TTGTCATCTA TATTGTCCTA 5267

ATAGTTTTCA GTCTGGCTTT ACGTAACTTT TACGGAAATT TCTAACATGT ACAAATGCCA 5327

TGTTCCTCCT TTCTTTCCTA CATGGCTGAA TTAGAAAACA AATTACTTCC ATTTTAAGTT 5387

TGGCTAAATT AGAAAACAAA TTACTACCAT TTTAAGTTTG GTGGCTAAAT AACGTGCTAA 5447

GGGAACATCT TAAAAAGTGA ATTTTGATCA AATATTTCTT AAGCATATGT GATAGACTTT 5507

GAAACCAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAA 5555

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1058 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1               5                  10                  15

Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
            20                  25                  30

Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
        35                  40                  45

Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
    50                  55                  60

Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
65                  70                  75                  80

Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
                85                  90                  95

Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
            100                 105                 110

Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
        115                 120                 125

Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
    130                 135                 140

Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
145                 150                 155                 160

Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
                165                 170                 175

Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
            180                 185                 190

Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
        195                 200                 205

Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
    210                 215                 220

Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
225                 230                 235                 240

Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
                245                 250                 255

Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
            260                 265                 270

Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
        275                 280                 285

Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
    290                 295                 300

Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
305                 310                 315                 320

Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
                325                 330                 335
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Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
340 345 350

Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
355 360 365

Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
370 375 380

Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
385 390 395 400

Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
405 410 415

Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
420 425 430

Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
435 440 445

Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
450 455 460

Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
465 470 475 480

Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
485 490 495

Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
500 505 510

Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
515 520 525

Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
530 535 540

Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
545 550 555 560

Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
565 570 575

Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
580 585 590

Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
595 600 605

Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
610 615 620

Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
625 630 635 640

His Ser Thr Leu Pro Gln His Ala Arg Thr Pro Leu Ile Ala Ala Gly
645 650 655

Val Ile Gly Gly Leu Phe Ile Leu Val Ile Val Gly Leu Thr Phe Ala
660 665 670

Val Tyr Val Arg Arg Lys Ser Ile Lys Lys Lys Arg Ala Leu Arg Arg
675 680 685

Phe Leu Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Thr Ala

690 695 700

Pro Asn Gln Ala Gln Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg
705 710 715 720

Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile
725 730 735

Trp Val Pro Glu Gly Glu Thr Val Lys Ile Pro Val Ala Ile Lys Ile
740 745 750

Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu
755 760 765

Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu
770 775 780

Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro
785 790 795 800

His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly
805 810 815

Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met
820 825 830

Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn
835 840 845

Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu
850 855 860

Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly
865 870 875 880

Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys
885 890 895

Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu
900 905 910

Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu
915 920 925

Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
930 935 940

Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
945 950 955 960

Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
965 970 975

Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
980 985 990

Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
995 1000 1005

Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
1010 1015 1020

Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
1025 1030 1035 1040

Ile Asp Ser Asn Arg Ser Val Arg Asn Asn Tyr Ile His Ile Ser Tyr
1045 1050 1055

Ser Phe

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3321 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 156..1782

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CATTAGCTGC AATTGATCAA GTGACTGAGA GAAGGGCAAC ATTCCATGCA ACAGTATAGT      60

GGTATGGAAA GCCCTGGATG TTGAAATCTA GCTTCAAAAA GCCTGTCTGG AAATGTAGTT     120

AATTGGATGA AGTGAGAAGA GATAAAACCA GAGAG GAA GCT CTG ATC ATG GCA        173
                                      Glu Ala Leu Ile Met Ala
                                        1               5

AGT ATG GAT CAT CCA CAC CTA GTC CGG TTG CTG GGT GTG TGT CTG AGC       221
Ser Met Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser
            10                  15                  20


CCA ACC ATC CAG CTG GTT ACT CAA CTT ATG CCC CAT GGC TGC CTG TTG       269
ro Thr Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu
        25                  30                  35

GAG TAT GTC CAC GAG CAC AAG GAT AAC ATT GGA TCA CAA CTG CTG CTT       317
Glu Tyr Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu
            45                  50

AAC TGG TGT GTC CAG ATA GCT AAG GGA ATG ATG TAC CTG GAA GAA AGA       365
Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg
        60                  65                  70

CGA CTC GTT CAT CGG GAT TTG GCA GCC CGT AAT GTC TTA GTG AAA TCT       413
Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser
    75                  80                  85

CCA AAC CAT GTG AAA ATC ACA GAT TTT GGG CTA GCC AGA CTC TTG GAA       461
Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu
 90                  95                  100

GGA GAT GAA AAA GAG TAC AAT GCT GAT GGA GGA AAG ATG CCA ATT AAA       509
Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys
                110                 115

TGG ATG GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT       557
Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser
            125                 130

GAC GTT TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA       605
Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly
        140                 145                 150
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GGA AAA CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA      653Gly
Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu
            160                 165

GAG AAA GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT      701
Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val
170                 175                 180

TAC ATG GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT      749
Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro
                190                 195

AAA TTT AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT      797
Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro
            205                 210

CAA AGA TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT      845
Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser
        220                 225                 230

CCA AAT GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG      893
Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu
    235                 240                 245

GAA GAT ATG ATG GAT GCT GAG GAG TAC TTG GTC CCT CAG GCT TTC AAC      941
Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn
250                 255                 260

ATC CCA CCT CCC ATC TAT ACT TCC AGA GCA AGA ATT GAC TCG AAT AGG      989
Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg
                270                 275

AGT GAA ATT GGA CAC AGC CCT CCT CCT GCC TAC ACC CCC ATG TCA GGA     1037
Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly
            285                 290


AAC CAG TTT GTA TAC CGA GAT GGA GGT TTT GCT GCT GAA CAA GGA GTG     1085
Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe Ala Ala Glu Gln Gly Val
        300                 305                 310

TCT GTG CCC TAC AGA GCC CCA ACT AGC ACA ATT CCA GAA GCT CCT GTG     1133
Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val
    315                 320                 325

GCA CAG GGT GCT ACT GCT GAG ATT TTT GAT GAC TCC TGC TGT AAT GGC     1181
Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly
330                 335                 340

ACC CTA CGC AAG CCA GTG GCA CCC CAT GTC CAA GAG GAC AGT AGC ACC     1229
Thr Leu Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr
                350                 355

CAG AGG TAC AGT GCT GAC CCC ACC GTG TTT GCC CCA GAA CGG AGC CCA     1277
Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro
            365                 370

CGA GGA GAG CTG GAT GAG GAA GGT TAC ATG ACT CCT ATG CGA GAC AAA     1325
Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys
        380                 385                 390

CCC AAA CAA GAA TAC CTG AAT CCA GTG GAG GAG AAC CCT TTT GTT TCT     1373
Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser
    395                 400                 405

CGG AGA AAA AAT GGA GAC CTT CAA GCA TTG GAT AAT CCC GAA TAT CAC
  1421
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Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His
            410                 415                 420

AAT GCA TCC AAT GGT CCA CCC AAG GCC GAG GAT GAG TAT GTG AAT GAG      1469
Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu
                430                 435

CCA CTG TAC CTC AAC ACC TTT GCC AAC ACC TTG GGA AAA GCT GAG TAC      1517
Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr
            445                 450

CTG AAG AAC AAC ATA CTG TCA ATG CCA GAG AAG GCC AAG AAA GCG TTT      1565
Leu Lys Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe
        460                 465                 470

GAC AAC CCT GAC TAC TGG AAC CAC AGC CTG CCA CCT CGG AGC ACC CTT      1613
Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu
    475                 480                 485

CAG CAC CCA GAC TAC CTG CAG GAG TAC AGC ACA AAA TAT TTT TAT AAA      1661
Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys
490                 495                 500

CAG AAT GGG CGG ATC CGG CCT ATT GTG GCA GAG AAT CCT GAA TAC CTC      1709
Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu
                510                 515

TCT GAG TTC TCC CTG AAG CCA GGC ACT GTG CTG CCG CCT CCA CCT TAC      1757
Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr
            525                 530

AGA CAC CGG AAT ACT GTG GTG TAAGCTCAGT TGTGGTTTTT TAGGTGGAGA         1808

Val
535                 540

GACACACCTG CTCCAATTTC CCCACCCCCC TCTCTTTCTC TGGTGGTCTT CCTTCTACCC

CCAGT AGTTTTGACA CTTCCCAGTG GAAGATACAG AGATGCAATG ATAGTTATGT        1928

GCTTACCTAA CTTGAACATT AGAGGGAAAG ACTGAAAGAG AAAGATAGGA GGAACCACAA   1988

TGTTTCTTCA TTTCTCTGCA TGGGTTGGTC AGGAGAATGA AACAGCTAGA GAAGGACCAG   2048

AAAATGTAAG GCAATGCTGC CTACTATCAA ACTAGCTGTC ACTTTTTTTC TTTTTCTTTT   2108

TCTTTCTTTG TTTCTTTCTT CCTCTTCTTT TTTTTTTTTT TTTTAAAGCA GATGGTTGAA   2168

ACACCCATGC TATCTGTTCC TATCTGCAGG AACTGATGTG TGCATATTTA GCATCCCTGG   2228

AAATCATAAT AAAGTTTCCA TTAGAACAAA AGAATAACAT TTTCTATAAC ATATGATAGT   2288

GTCTGAAATT GAGAATCCAG TTTCTTTCCC CAGCAGTTTC TGTCCTAGCA AGTAAGAATG   2348

GCCAACTCAA CTTTCATAAT TTAAAAATCT CCATTAAAGT TATAACTAGT AATTATGTTT   2408

TCAACACTTT TTGGTTTTTT TCATTTTGTT TTGCTCTGAC CGATTCCTTT ATATTTGCTC   2468

CCCTATTTTT GGCTTTAATT TCTAATTGCA AAGATGTTTA CATCAAAGCT TCTTCACAGA   2528

ATTTAAGCAA GAAATATTTT AATATAGTGA AATGGCCACT ACTTTAAGTA TACAATCTTT   2588

AAAATAAGAA AGGGAGGCTA ATATTTTTCA TGCTATCAAA TTATCTTCAC CCTCATCCTT   2648

TACATTTTTC AACATTTTTT TTTCTCCATA AATGACACTA CTTGATAGGC CGTTGGTTGT   2708
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CTGAAGAGTA GAAGGGAAAC TAAGAGACAG TTCTCTGTGG TTCAGGAAAA CTACTGATAC 2768

TTTCAGGGGT GGCCCAATGA GGGAATCCAT TGAACTGGAA GAAACACACT GGATTGGGTA 2828

TGTCTACCTG GCAGATACTC AGAAATGTAG TTTGCACTTA AGCTGTAATT TTATTTGTTC 2888

TTTTTCTGAA CTCCATTTTG GATTTTGAAT CAAGCAATAT GGAAGCAACC AGCAAATTAA 2948

CTAATTTAAG TACATTTTTA AAAAAAGAGC TAAGATAAAG ACTGTGGAAA TGCCAAACCA 3008

AGCAAATTAG GAACCTTGCA ACGGTATCCA GGGACTATGA TGAGAGGCCA GCACATTATC 3068

TTCATATGTC ACCTTTGCTA CGCAAGGAAA TTTGTTCAGT TCGTATACTT CGTAAGAAGG 3128

AATGCGAGTA AGGATTGGCT TGAATTCCAT GGAATTTCTA GTATGAGACT ATTTATATGA 3188

AGTAGAAGGT AACTCTTTGC ACATAAATTG GTATAATAAA AAGAAAAACA CAAACATTCA 3248

AAGCTTAGGG ATAGGTCCTT GGGTCAAAAG TTGTAAATAA ATGTGAAACA TCTTCTCAAA 3308

AAAAAAAAAA AAA 3321

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 541 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu
1 5 10 15

Leu Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met
20 25 30

Pro His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile
35 40 45

Gly Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met
50 55 60

Met Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg
65 70 75 80

Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly
85 90 95

Leu Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly
100 105 110

Gly Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg
115 120 125

Lys Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp
130 135 140

Glu Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg
145 150 155 160

Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro
165 170 175

Ile Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile

180 185 190

Asp Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser
195 200 205

Arg Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp
210 215 220

Arg Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu
225 230 235 240

Leu Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu
245 250 255

Val Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala
260 265 270

Arg Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala
275 280 285

Tyr Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe
290 295 300

Ala Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr
305 310 315 320

Ile Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp
325 330 335

Asp Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val
340 345 350

Gln Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe
355 360 365

Ala Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met
370 375 380

Thr Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu
385 390 395 400

Glu Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu
405 410 415

Asp Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu
420 425 430

Asp Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr
435 440 445

Leu Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu
450 455 460

Lys Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu
465 470 475 480

Pro Pro Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser
485 490 495

Thr Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala
500 505 510

Glu Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val
515 520 525

Leu Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
530 535 540

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1210 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
1 5 10 15

Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
20 25 30

Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
35 40 45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
50 55 60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
100 105 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
115 120 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
130 135 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145 150 155 160

Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
165 170 175

Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
180 185 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
195 200 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
210 215 220

Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225 230 235 240

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
245 250 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
260 265 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly

275 280 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
290 295 300

Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305 310 315 320

Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
325 330 335

Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
340 345 350

Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
355 360 365

Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
370 375 380

Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385 390 395 400

Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
405 410 415

Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
420 425 430

His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
435 440 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
450 455 460

Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465 470 475 480

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
485 490 495

Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
500 505 510

Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
515 520 525

Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Lys Leu Leu Glu Gly
530 535 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545 550 555 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
610 615 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625 630 635 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
645 650 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
660 665 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
675 680 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
690 695 700

Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705 710 715 720

Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
725 730 735

Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
740 745 750

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
755 760 765

Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
770 775 780

Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785 790 795 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
805 810 815

Trp Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Asp Arg Arg
820 825 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
835 840 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
850 855 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865 870 875 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser
885 890 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly
900 905 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
915 920 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
930 935 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945 950 955 960

Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
965 970 975

Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
980 985 990

Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
995 1000 1005

Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
1010 1015 1020

Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025 1030 1035 1040

Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
1045 1050 1055

Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
1060 1065 1070

Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
1075 1080 1085

Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
1090 1095 1100

Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105 1110 1115 1120

Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
1125 1130 1135

Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
1140 1145 1150

Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
1155 1160 1165

Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
1170 1175 1180

Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
1185 1190 1195 1200

Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
1205 1210

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1255 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1               5                  10                  15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30

Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285
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Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
290 295 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
355 360 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
370 375 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385 390 395 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
405 410 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
420 425 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
435 440 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
450 455 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465 470 475 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
485 490 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
500 505 510

Gln Leu Cys Ala Arg Arg Ala Leu Leu Gly Ser Gly Pro Thr Gln Cys
515 520 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
530 535 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545 550 555 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
565 570 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
580 585 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
595 600 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
610 615 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys

625 630 635 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Val Ser
645 650 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
660 665 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
675 680 685

Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
690 695 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705 710 715 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
725 730 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
740 745 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
755 760 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
770 775 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785 790 795 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
805 810 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
820 825 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
835 840 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
850 855 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865 870 875 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
885 890 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
900 905 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
915 920 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
930 935 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945 950 955 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
965 970 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu

980 985 990

Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
995 1000 1005

Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
1010 1015 1020

Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025 1030 1035 1040

Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
1045 1050 1055

Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
1060 1065 1070

Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
1075 1080 1085

Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
1090 1095 1100

Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105 1110 1115 1120

Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
1125 1130 1135

Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
1140 1145 1150

Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
1155 1160 1165

Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
1170 1175 1180

Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185 1190 1195 1200

Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
1205 1210 1215

Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
1220 1225 1230

Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Val Ala Glu Asn Pro Glu
1235 1240 1245

Tyr Gly Leu Asp Val Pro Val
1250 1255

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1342 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
1 5 10 15

la Arg Gly Ser Glu Val Gly Asn Ser Gln Ala Val Cys Pro Gly Thr
20 25 30

Leu Asn Gly Leu Ser Val Thr Gly Asp Ala Glu Asn Gln Tyr Gln Thr
35 40 45

Leu Tyr Lys Leu Tyr Glu Arg Cys Glu Val Val Met Gly Asn Leu Glu
50 55 60

Ile Val Leu Thr Gly His Asn Ala Asp Leu Ser Phe Leu Gln Trp Ile
65 70 75 80

Arg Glu Val Thr Gly Tyr Val Leu Val Ala Met Asn Glu Phe Ser Thr
85 90 95

Leu Pro Leu Pro Asn Leu Arg Val Val Arg Gly Thr Gln Val Tyr Asp
100 105 110

Gly Lys Phe Ala Ile Phe Val Met Leu Asn Tyr Asn Thr Asn Ser Ser
115 120 125

His Ala Leu Arg Gln Leu Arg Leu Thr Gln Leu Thr Glu Ile Leu Ser
130 135 140

Gly Gly Val Tyr Ile Glu Lys Asn Asp Lys Leu Cys His Met Asp Thr
145 150 155 160

Ile Asp Trp Arg Asp Ile Val Arg Asp Arg Asp Ala Glu Ile Val Val
165 170 175

Lys Asp Asn Gly Arg Ser Cys Pro Pro Cys His Glu Val Cys Lys Gly
180 185 190

Arg Cys Trp Gly Pro Gly Ser Glu Asp Cys Gln Thr Leu Thr Lys Thr
195 200 205

Ile Cys Ala Pro Gln Cys Asn Gly His Cys Phe Gly Pro Asn Pro Asn
210 215 220

Gln Cys Cys His Asp Glu Cys Ala Gly Gly Cys Ser Gly Pro Gln Asp
225 230 235 240

Thr Asp Cys Phe Ala Cys Arg His Phe Asn Asp Ser Gly Ala Cys Val
245 250 255

Pro Arg Cys Pro Gln Pro Leu Val Tyr Asn Lys Leu Thr Phe Gln Leu
260 265 270

Glu Pro Asn Pro His Thr Lys Tyr Gln Tyr Gly Gly Val Cys Val Ala
275 280 285

Ser Cys Pro His Asn Phe Val Val Asp Gln Thr Ser Cys Val Arg Ala
290 295 300

Cys Pro Pro Asp Lys Met Glu Val Asp Lys Asn Gly Leu Lys Met Cys
305 310 315 320

Glu Pro Cys Gly Gly Leu Cys Pro Lys Ala Cys Glu Gly Thr Gly Ser
325 330 335

Gly Ser Arg Phe Gln Thr Val Asp Ser Ser Asn Ile Asp Gly Phe Val
340 345 350

Asn Cys Thr Lys Ile Leu Gly Asn Leu Asp Phe Leu Ile Thr Gly Leu
355 360 365

Asn Gly Asp Pro Trp His Lys Ile Pro Ala Leu Asp Pro Glu Lys Leu
370 375 380

Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly Tyr Leu Asn Ile Gln
385 390 395 400

Ser Trp Pro Pro His Met His Asn Phe Ser Val Phe Ser Asn Leu Thr
405 410 415

Thr Ile Gly Gly Arg Ser Leu Tyr Asn Arg Gly Phe Ser Leu Leu Ile
420 425 430

Met Lys Asn Leu Asn Val Thr Ser Leu Gly Phe Arg Ser Leu Lys Glu
435 440 445

Ile Ser Ala Gly Arg Ile Tyr Ile Ser Ala Asn Arg Gln Leu Cys Tyr
450 455 460

His His Ser Leu Asn Trp Thr Lys Val Leu Arg Gly Pro Thr Glu Glu
465 470 475 480

Arg Leu Asp Ile Lys His Asn Arg Pro Arg Arg Asp Cys Val Ala Glu
485 490 495

Gly Lys Val Cys Asp Pro Leu Cys Ser Ser Gly Gly Cys Trp Gly Pro
500 505 510

Gly Pro Gly Gln Cys Leu Ser Cys Arg Asn Tyr Ser Arg Gly Gly Val
515 520 525

Cys Val Thr His Cys Asn Phe Leu Asn Gly Glu Pro Arg Glu Phe Ala
530 535 540

His Glu Ala Glu Cys Phe Ser Cys His Pro Glu Cys Gln Pro Met Gly
545 550 555 560

Gly Thr Ala Thr Cys Asn Gly Ser Gly Ser Asp Thr Cys Ala Gln Cys
565 570 575

Ala His Phe Arg Asp Gly Pro His Cys Val Ser Ser Cys Pro His Gly
580 585 590

Val Leu Gly Ala Lys Gly Pro Ile Tyr Lys Tyr Pro Asp Val Gln Asn
595 600 605

Glu Cys Arg Pro Cys His Glu Asn Cys Thr Gln Gly Cys Lys Gly Pro
610 615 620

Glu Leu Gln Asp Cys Leu Gly Gln Thr Leu Val Leu Ile Gly Lys Thr
625 630 635 640

His Leu Thr Met Ala Leu Thr Val Ile Ala Gly Leu Val Val Ile Phe
645 650 655

Met Met Leu Gly Gly Thr Phe Leu Tyr Trp Arg Gly Arg Arg Ile Gln
660 665 670

Asn Lys Arg Ala Met Arg Arg Tyr Leu Glu Arg Gly Glu Ser Ile Glu
675 680 685

Pro Leu Asp Pro Ser Glu Lys Ala Asn Lys Val Leu Ala Arg Ile Phe
690 695 700

Lys Glu Thr Glu Leu Arg Lys Leu Lys Val Leu Gly Ser Gly Val Phe
705 710 715 720

Gly Thr Val His Lys Gly Val Trp Ile Pro Glu Gly Glu Ser Ile Lys
725 730 735

le Pro Val Cys Ile Lys Val Ile Glu Asp Lys Ser Gly Arg Gln Ser
740 745 750

Phe Gln Ala Val Thr Asp His Met Leu Ala Ile Gly Ser Leu Asp His
755 760 765

Ala His Ile Val Arg Leu Leu Gly Leu Cys Pro Gly Ser Ser Leu Gln
770 775 780

Leu Val Thr Gln Tyr Leu Pro Leu Gly Ser Leu Leu Asp His Val Arg
785 790 795 800

Gln His Arg Gly Ala Leu Gly Pro Gln Leu Leu Leu Asn Trp Gly Val
805 810 815

Gln Ile Ala Lys Gly Met Tyr Tyr Leu Glu Glu His Gly Met Val His
820 825 830

Arg Asn Leu Ala Ala Arg Asn Val Leu Leu Lys Ser Pro Ser Gln Val
835 840 845

Gln Val Ala Asp Phe Gly Val Ala Asp Leu Leu Pro Pro Asp Asp Lys
850 855 860

Gln Leu Leu Tyr Ser Glu Ala Lys Thr Pro Ile Lys Trp Met Ala Leu
865 870 875 880

Glu Ser Ile His Phe Gly Lys Tyr Thr His Gln Ser Asp Val Trp Ser
885 890 895

Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ala Glu Pro Tyr
900 905 910

Ala Gly Leu Arg Leu Ala Glu Val Pro Asp Leu Leu Glu Lys Gly Glu
915 920 925

Arg Leu Ala Gln Pro Gln Ile Cys Thr Ile Asp Val Tyr Met Val Met
930 935 940

Val Lys Cys Trp Met Ile Asp Glu Asn Ile Arg Pro Thr Phe Lys Glu
945 950 955 960

Leu Ala Asn Glu Phe Thr Arg Met Ala Arg Asp Pro Pro Arg Tyr Leu
965 970 975

Val Ile Lys Arg Glu Ser Gly Pro Gly Ile Ala Pro Gly Pro Glu Pro
980 985 990

His Gly Leu Thr Asn Lys Lys Leu Glu Glu Val Glu Leu Glu Pro Glu
995 1000 1005

Leu Asp Leu Asp Leu Asp Leu Glu Ala Glu Glu Asp Asn Leu Ala Thr
1010 1015 1020

Thr Thr Leu Gly Ser Ala Leu Ser Leu Pro Val Gly Thr Leu Asn Arg
1025 1030 1035 1040

Pro Arg Gly Ser Gln Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro
1045 1050 1055

Met Asn Gln Gly Asn Leu Gly Gly Ser Cys Gln Glu Ser Ala Val Ser
1060 1065 1070

Gly Ser Ser Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro
1075 1080 1085

Arg Gly Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser
1090 1095 1100

Glu Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1105 1110 1115 1120

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln Arg
1125 1130 1135

His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly Leu Glu
1140 1145 1150

Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His Leu Lys Gly
1155 1160 1165

Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val Gly Leu Ser Ser
1170 1175 1180

Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu Glu Tyr Glu Tyr Met
1185 1190 1195 1200

Asn Arg Arg Arg Arg His Ser Pro Pro His Pro Pro Arg Pro Ser Ser
1205 1210 1215

Leu Glu Glu Leu Gly Tyr Glu Tyr Met Asp Val Gly Ser Asp Leu Ser
1220 1225 1230

Ala Ser Leu Gly Ser Thr Gln Ser Cys Pro Leu His Pro Val Pro Ile
1235 1240 1245

Met Pro Thr Ala Gly Thr Thr Pro Asp Glu Asp Tyr Glu Tyr Met Asn
1250 1255 1260

Arg Gln Arg Asp Gly Gly Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly
1265 1270 1275 1280

Ala Cys Pro Ala Ser Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln
1285 1290 1295

Gly Pro Gly His Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr
1300 1305 1310

Leu Arg Ser Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr
1315 1320 1325

Trp His Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1330 1335 1340

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 911 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1               5                  10                  15

Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
            20                  25                  30

Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
        35                  40                  45

Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
    50                  55                  60

Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
65                  70                  75                  80

Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
                85                  90                  95

Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
            100                 105                 110

Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
        115                 120                 125

Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
    130                 135                 140

Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
145                 150                 155                 160

Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
                165                 170                 175

Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
            180                 185                 190

Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
        195                 200                 205

Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
    210                 215                 220

Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
225                 230                 235                 240

Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
                245                 250                 255

Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
            260                 265                 270

Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
        275                 280                 285

Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
    290                 295                 300

Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
```

305 310 315 320

Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
325 330 335

le Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
340 345 350

Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
355 360 365

Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
370 375 380

Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
385 390 395 400

Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
405 410 415

Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
420 425 430

Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
435 440 445

Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
450 455 460

Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
465 470 475 480

Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
485 490 495

Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
500 505 510

Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
515 520 525

Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
530 535 540

Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
545 550 555 560

Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
565 570 575

Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
580 585 590

Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
595 600 605

Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
610 615 620

Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
625 630 635 640

His Ser Thr Leu Pro Gln Asp Pro Val Lys Val Lys Ala Leu Glu Gly
645 650 655

Phe Pro Arg Leu Val Gly Pro Asp Phe Phe Gly Cys Ala Glu Pro Ala
660 665 670

Asn Thr Phe Leu Asp Pro Glu Glu Pro Lys Ser Cys Asp Lys Thr His
675 680 685

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
690 695 700

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
705 710 715 720

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
725 730 735

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Val Ala Lys
740 745 750

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
755 760 765

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
770 775 780

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
785 790 795 800

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
805 810 815

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
820 825 830

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
835 840 845

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
850 855 860

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
865 870 875 880

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
885 890 895

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
900 905 910

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Xaa Gly Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Leu Ala Ala Arg Asn
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Ile Lys Trp Met Ala
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACNGTNTGGG ARYTNAYHAC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAYGTNAARA THACNGAYTT YGG 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GACGAATTCC NATHAARTGG ATGGC 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACAYTTNARD ATDATCATRT ANAC 24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AANGTCATNA RYTCCCA 17

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCCAGNGCGA TCCAYTTDAT NGG 23

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGRTCDATCA TCCARCCT 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTGCTGTCAG CATCGATCAT 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Thr Val Trp Glu Leu Met Thr
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Lys Ile Thr Asp Phe Gly
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Val Tyr Met Ile Ile Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Trp Glu Leu Met Thr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Ile Lys Trp Met Ala Leu Glu
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Trp Met Ile Asp Pro
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GACTCGAGTC GACATCGATT TTTTTTTTTT TTTTT 35

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAAGAAAGAC GACTCGTTCA TCGG 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GACCATGACC ATGTAAACGT CAATA 25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Leu Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly
1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 3
(D) OTHER INFORMATION: "Xaa = Any amino acid"

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 6
(D) OTHER INFORMATION: "Xaa = Any amino acid"

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 7
(D) OTHER INFORMATION: "Xaa = Any amino acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Gly Xaa Lys Pro Xaa Xaa Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGAAGCTTC TAGAGATCCC TCGAC 25

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTTTTTACCT TTTTTATCTT CTTTGTGTTC GGTTGTGTAT TTCACACGCC 50

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CAAAAATGGA AAAAATAGAA GAAACAGAAG CCATCTCATAA AGTGTGCGG 50

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTCTTTTTC GCCTCCTTGA GATGATTAGA TCTCTG 36

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GTCAGAGTTC ATATGGTAGT TAAGCCCCCC CAAAAC 36

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CAAAGATCCT CTAAGCTTGT AGAGTTCCTC CGATTTGTAA AAAGATGCCA TAACATAGTT 60

CTGGCAACGG TCGCCAGTAA ATTCGTTCGG GCACTTGCAC AAGTATCTTG ACGG 94

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Val Val Lys Pro Pro Gln Asn Lys Thr Glu Ser Glu Asn Thr Ser
1 5 10 15

Asp Lys Pro Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg
20 25 30

Arg Asn Arg Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe
35 40 45

Cys Val Asn Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro
50 55 60

Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys
65 70 75 80

Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr
85 90 95

INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1389 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1386

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

ATG GTA GTT AAG CCC CCC CAA AAC AAG ACG GAA AGT GAA AAT ACT TCA 48
Met Val Val Lys Pro Pro Gln Asn Lys Thr Glu Ser Glu Asn Thr Ser
1 5 10 15

GAT AAA CCC AAA AGA AAG AAA AAG GGA GGC AAA AAT GGA AAA AAT AGA 96
Asp Lys Pro Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg
20 25 30

AGA AAC AGA AGC CAT CTC ATA AAG TGT GCG GAG AAG GAG AAA ACT TTC 144
Arg Asn Arg Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe
35 40 45

TGT GTG AAT GGG GGC GAG TGC TTC ACG GTG AAG GAC CTG TCA AAC CCG 192

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Cys Val Asn Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro
     50                  55                  60

TCA AGA TAC TTG TGC AAG TGC CCG AAC GAA TTT ACT GGC GAC CGT TGC      240
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys
 65                  70                  75                  80

CAG AAC TAT GTT ATG GCA TCT TTT TAC AAA GCG GAG GAA CTC TAC AAG      288
Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Lys
                 85                  90                  95

CTT ATG GCC GAG GAA GGC GGC AGC CTG GCC GCG CTG ACC GCG CAC CAG      336
Leu Met Ala Glu Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
            100                 105                 110

GCT TGC CAC CTG CCG CTG GAG ACT TTC ACC CGT CAT CGC CAG CCG CGC      384
Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        115                 120                 125

GGC TGG GAA CAA CTG GAG CAG TGC GGC TAT CCG GTG CAG CGG CTG GTC      432
Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    130                 135                 140

GCC CTC TAC CTG GCG GCG CGG CTG TCG TGG AAC CAG GTC GAC CAG GTG      480
Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
145                 150                 155                 160

ATC CGC AAC GCC CTG GCC AGC CCC GGC AGC GGC GGC GAC CTG GGC GAA      528
Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                165                 170                 175

GCG ATC CGC GAG CAG CCG GAG CAG GCC CGT CTG GCC CTG ACC CTG GCC      576
Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            180                 185                 190

GCC GCC GAG AGC GAG CGC TTC GTC CGG CAG GGC ACC GGC AAC GAC GAG      624
Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        195                 200                 205

GCC GGC GCG GCC AAC GCC GAC GTG GTG AGC CTG ACC TGC CCG GTC GCC      672
Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    210                 215                 220

GCC GGT GAA TGC GCG GGC CCG GCG GAC AGC GGC GAC GCC CTG CTG GAG      720
Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
225                 230                 235                 240

CGC AAC TAT CCC ACT GGC GCG GAG TTC CTC GGC GAC GGC GGC GAC GTC      768
Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                245                 250                 255

AGC TTC AGC ACC CGC GGC ACG CAG AAC TGG ACG GTG GAG CGG CTG CTC      816
Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            260                 265                 270

CAG GCG CAC CGC CAA CTG GAG GAG CGC GGC TAT GTG TTC GTC GGC TAC      864
Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        275                 280                 285

CAC GGC ACC TTC CTC GAA GCG GCG CAA AGC ATC GTC TTC GGC GGG GTG      912
His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    290                 295                 300

CGC GCG CGC AGC CAG GAC CTC GAC GCG ATC TGG CGC GGT TTC TAT ATC      960
Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
305                 310                 315                 320
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GCC GGC GAT CCG GCG CTG GCC TAC GGC TAC GCC CAG GAC CAG GAA CCC      1008
Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
                325                 330                 335

GAC GCA CGC GGC CGG ATC CGC AAC GGT GCC CTG CTG CGG GTC TAT GTG      1056
Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
            340                 345                 350

CCG CGC TCG AGC CTG CCG GGC TTC TAC CGC ACC AGC CTG ACC CTG GCC      1104
Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        355                 360                 365

GGC GGC GAG GCG GCG GGC GAG GTC GAA CGG CTG ATC GGC CAT CCG CTG      1152
Gly Gly Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    370                 375                 380

CCG CTG CGC CTG GAC GCC ATC ACC GGC CCC GAG GAG GAA GGC GGG CGC      1200
Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
385                 390                 395                 400

CTG GAG ACC ATT CTC GGC TGG CCG CTG GCC GAG CGC ACC GTG GTG ATT      1248
Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                405                 410                 415

CCC TCG GCG ATC CCC ACC GAC CCG CGC AAC GTC GGC GGC GAC CTC GAC      1296
Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            420                 425                 430

CCG TCC AGC ATC CCC GAC AAG GAA CAG GCG ATC AGC GCC CTG CCG GAC      1344
Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        435                 440                 445

TAC GCC AGC CAG CCC GGC AAA CCG CCG CGC GAG GAC CTG AAG               1386
Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys
    450                 455                 460

TAA
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

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Met Val Val Lys Pro Pro Gln Asn Lys Thr Glu Ser Glu Asn Thr Ser
  1               5                  10                  15

Asp Lys Pro Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg
            20                  25                  30

Arg Asn Arg Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe
        35                  40                  45

Cys Val Asn Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro
    50                  55                  60

Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys
65                  70                  75                  80

Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Lys
                85                  90                  95

Leu Met Ala Glu Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
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            100                 105                 110

Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        115                 120                 125

Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    130                 135                 140

Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
145                 150                 155                 160

Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                165                 170                 175

Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            180                 185                 190

Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        195                 200                 205

Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    210                 215                 220

Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
225                 230                 235                 240

Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                245                 250                 255

Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            260                 265                 270

Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        275                 280                 285

His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    290                 295                 300

Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
305                 310                 315                 320

Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
                325                 330                 335

Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
            340                 345                 350

Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        355                 360                 365

Gly Gly Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    370                 375                 380

Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
385                 390                 395                 400

Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                405                 410                 415

Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            420                 425                 430

Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        435                 440                 445

Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys
    450                 455                 460
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WHAT IS CLAIMED IS:

1. A recombinant polynucleotide comprising a sequence of at least about 200 nucleotides having greater than 80% homology to a contiguous portion of the HER4 nucleotide sequence depicted in FIG. 1A and 1B or its complement.

2. A recombinant polynucleotide comprising a sequence of nucleotides encoding at least about 70 contiguous amino acids within the HER4 amino acid sequence depicted in FIG. 1A and 1B.

3. A recombinant polynucleotide comprising a contiguous sequence of at least about 200 nucleotides within the HER4 nucleotide coding sequence depicted in FIG. 1A and 1B or its complement.

4. A recombinant polynucleotide comprising the HER4 nucleotide coding sequence depicted in FIG. 1A and 1B or its complement.

5. A recombinant polynucleotide according to claim 1, 2, 3, or 4 which is a DNA polynucleotide.

6. A recombinant polynucleotide according to claim 1, 2, 3, or 4 which is a RNA polynucleotide.

7. An assay kit comprising a recombinant polynucleotide according to claim 1, 2, 3, or 4 to which a detectable label has been added.

8. A polymerase chain reaction kit (PCR) comprising a pair of primers capable of priming cDNA synthesis in a PCR reaction, wherein each primer is a polynucleotide according to claim 5.

9. The PCR kit according to claim 8 further comprising a polynucleotide probe capable of hybridizing to a region of the HER4 gene between and not including the nucleotide sequences to which the primers hybridize.

10. A polypeptide comprising a sequence of at least about 80 amino acids having greater than 90% identity to a contiguous portion of the HER4 amino acid sequence depicted in FIG. 1A and 1B.

11. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 1A and 1B from amino acid residues 1 through 1308.

12. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 1A and 1B from amino acid residues 26 through 1308.

13. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 1A and 1B from amino acid residues 1 through 1045.

14. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 1A and 1B from amino acid residues 26 through 1045.

15. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 2A and 2B.

16. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 1A and 1B from amino acid residues 772 through 1308.

17. A HER4 polypeptide comprising the amino acid sequence depicted in FIG. 3.

18. An antibody capable of inhibiting the interaction of a soluble polypeptide and human HER4.

19. An antibody according to claim 18 wherein the soluble polypeptide is a heregulin.

20. An antibody capable of stimulating HER4 tyrosine autophosphorylation.

21. An antibody capable of inducing a HER4-mediated signal in a cell, which signal results in modulation of growth or differentiation of the cell.

22. An antibody capable of inhibiting HepG2 fraction 17-stimulated tyrosine phosphorylation of HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC.

23. An antibody which immunospecifically binds to human HER4.

24. An antibody according to claim 23 which resides on the cell surface after binding to HER4.

25. An antibody according to claim 23 which is internalized into the cell after binding to HER4.

26. An antibody which immunospecifically binds to human HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC.

27. An antibody according to claim 23 which neutralizes HER4 biological activity.

28. An antibody according to claim 23 which is conjugated to a drug or toxin.

29. An antibody according to claim 23 which is radiolabeled.

30. Plasmid pBSHER4Y as deposited with the ATCC.

31. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to claim 10, 11, 12, 13, 14, 15, 16, or 17.

32. A host cell transfected with a recombinant vector according to claim 31.

33. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to claim 10, 11, 12, 13, 14, 15, 16, or 17, wherein the coding sequence is operably linked to a control sequence which is capable of directing the expression of the coding sequence in a host cell transfected therewith.

34. A host cell transfected with a recombinant vector according to claim 33.

35. Cell line CHO/HER4 21-2 as deposited with the ATCC.

36. An assay for detecting the presence of a HER4 ligand in a sample comprising:

(a) applying the sample to cells which have been engineered to overexpress HER4; and

(b) detecting an ability of the ligand to affect an activity mediated by HER4.

37. The assay according to claim 36, wherein the cells are CHO/HER4 21-2 cells as deposited with the ATCC.

38. The assay according to claim 36, wherein the activity detected is HER4 tyrosine phosphorylation.

39. The assay according to claim 36, wherein the activity detected is morphologic differentiation.

40. A ligand for HER4 comprising a polypeptide which binds to HER4, stimulates tyrosine phosphorylation of HER4, and affects a biological activity mediated by HER4.

41. A ligand according to claim 40 which is capable of inducing morphological differentiation when added to cultured MDA-MB-453 cells.

42. A ligand according to claim 40 obtained from cultured HepG2 cell conditioned media.

43. An immunoassay for detecting HER4 comprising:

(a) providing an antibody according to claim 23 or 26;

(b) incubating a biological sample with the antibody under conditions which allow for the binding of the antibody to HER4; and

(c) determining the amount of antibody present as a HER4-antibody complex.

44. A method for the *in vivo* delivery of a drug or toxin to cells expressing HER4 comprising conjugating an antibody according to claim 23 or 26, or an active fragment thereof, to the drug or toxin, and delivering the resulting conjugate to an individual by using a formulation, dose, and route of administration such that the conjugate binds to HER4.

45. A HER4 ligand comprising a polypeptide which is capable of binding to HER4 and activating protein kinase activity.

46. The ligand of claim 40 or claim 45 which is heregulin.

47. The ligand of claim 45 which is p45.

48. An isolated polypeptide of molecular weight 45 kDa as determined by SDS-Page analysis having an N-terminal amino acid sequence Ser-Gly-X-Lys-Pro-X-X-Ala-Ala, wherein said polypeptide is capable of binding to HER4 as expressed in MDA-MB-453 cells.

49. A chimeric polypeptide comprising a HER4 ligand fused to a cytotoxin.

50. A chimeric polypeptide according to claim 49 wherein the HER4 ligand is a heregulin, a functional derivative of a heregulin, or a homolog of a heregulin, which is capable of binding to and activating HER4.

51. A chimeric polypeptide according to claim 49 or 50 wherein the heregulin is heregulin-α (HRG-α).

52. A chimeric polypeptide according to claim 49 or 50 wherein the heregulin is heregulin-β1 (HRG-β1).

53. A chimeric polypeptide according to claim 49 or 50 wherein the heregulin is heregulin-β2 (HRG-β2).

54. A chimeric polypeptide according to claim 53 further comprising the amphiregulin leader peptide at the amino terminus.

55. A chimeric polypeptide according to claim 49 or 50 wherein the heregulin is heregulin-β3 (HRG-β3).

56. A chimeric polypeptide according to claim 49, 50, or 54 wherein the cytotoxin is PE40 or a functionally equivalent *Pseudomonas arabinosa* exotoxin derivative.

57. HAR-TX β2 having the amino acid sequence depicted in SEQ ID No:42.

58. A recombinant polynucleotide comprising a sequence of nucleotides encoding a chimeric polypeptide according to claim 49.

59. A recombinant polynucleotide comprising a sequence of nucleotides encoding HAR-TX β2.

60. A recombinant vector comprising the polynucleotide according to claim 59 under the control of an IPTG-inducible T7-promoter.

61. A monoclonal antibody which competitively inhibits the immunospecific binding of the monoclonal

antibody produced by hybridoma cell line 6-4-11 as deposited with the ATCC to its epitope.

62. A monoclonal antibody which competitively inhibits the immunospecific binding of the monoclonal antibody produced by hybridoma cell line 7-142 as deposited with the ATCC to its epitope.

63. Hybridoma cell line 6-4-11 as deposited with the ATCC and assigned accession number HB11715.

64. Hybridoma cell line 7-142 as deposited with the ATCC and assigned accession number HB11716.

65. A method of delivering a molecule to a cell expressing HER4, comprising:

(a) generating a conjugate or a fusion of the molecule and a HER4 ligand; and

(b) contacting the cell with the conjugate or fusion such that it binds to HER4 and is thereby internalized into the cell.

66. A method of delivering a molecule to a cell which expresses HER4, comprising contacting the cell with a conjugate or a fusion of a HER4 ligand and the molecule.

67. The method according to claim 65 or 66 wherein the molecule is a polypeptide.

68. The method according to claim 65 or 66 wherein the molecule is a polynucleotide.

69. The method according to claim 65 or 66 wherein the molecule is a radionuclide.

70. The method according to claim 65 or 66 wherein the molecule is an imaging label.

71. A method of delivering a cytotoxin to the cytoplasm of a cell which expresses HER4, comprising contacting the cell with a conjugate of the cytotoxin and a HER4 ligand, such that the conjugate binds to, activates, and is internalized via HER4.

72. A method of delivering a cytotoxin to the cytoplasm of a cell which expresses HER4, comprising contacting the cell with a chimeric polypeptide comprising a HER4 ligand fused to the cytotoxin, such that the chimeric polypeptide binds to, activates, and is internalized via HER4.

73. The method according to claim 72 wherein the chimeric polypeptide is HAR-TX β2.

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   1                                                MetLysProAlaThrGlyLeuTrpValTrp
   1 AATTGTCAGCACGGGATCTGAGACTTCCAAAAAATGAAGCCGGCGACAGGACTTTGGGTCTGG

  11 ValSerLeuLeuValAlaAlaGlyThrValGlnProSerAspSerGlnSerValCysAlaGly
  64 GTGAGCCTTCTCGTGGCGGCGGGGACCGTCCAGCCCAGCGATTCTCAGTCAGTGTGTGCAGGA

  32 ThrGluAsnLysLeuSerSerLeuSerAspLeuGluGlnGlnTyrArgAlaLeuArgLysTyr
 127 ACGGAGAATAAACTGAGCTCTCTCTCTGACCTGGAACAGCAGTACCGAGCCTTGCGCAAGTAC

  53 TyrGluAsnCysGluValValMetGlyAsnLeuGluIleThrSerIleGluHisAsnArgAsp
 190 TATGAAAACTGTGAGGTTGTCATGGGCAACCTGGAGATAACCAGCATTGAGCACAACCGGGAC

  74 LeuSerPheLeuArgSerValArgGluValThrGlyTyrValLeuValAlaLeuAsnGlnPhe
 253 CTCTCCTTCCTGCGGTCTGTTCGAGAAGTCACAGGCTACGTGTTAGTGGCTCTTAATCAGTTT

  95 ArgTyrLeuProLeuGluAsnLeuArgIleIleArgGlyThrLysLeuTyrGluAspArgTyr
 316 CGTTACCTGCCTCTGGAGAATTTACGCATTATTCGTGGGACAAAACTTTATGAGGATCGATAT

 116 AlaLeuAlaIlePheLeuAsnTyrArgLysAspGlyAsnPheGlyLeuGlnGluLeuGlyLeu
 379 GCCTTGGCAATATTTTTAAACTACAGAAAAGATGGAAACTTTGGACTTCAAGAACTTGGATTA

 137 LysAsnLeuThrGluIleLeuAsnGlyGlyValTyrValAspGlnAsnLysPheLeuCysTyr
 442 AAGAACTTGACAGAAATCCTAAATGGTGGAGTCTATGTAGACCAGAACAAATTCCTTTGTTAT

 158 AlaAspThrIleHisTrpGlnAspIleValArgAsnProTrpProSerAsnLeuThrLeuVal
 505 GCAGACACCATTCATTGGCAAGATATTGTTCGGAACCCATGGCCTTCCAACTTGACTCTTGTG

 179 SerThrAsnGlySerSerGlyCysGlyArgCysHisLysSerCysThrGlyArgCysTrpGly
 568 TCAACAAATGGTAGTTCAGGATGTGGACGTTGCCATAAGTCCTGTACTGGCCGTTGCTGGGGA

 200 ProThrGluAsnHisCysGlnThrLeuThrArgThrValCysAlaGluGlnCysAspGlyArg
 631 CCCACAGAAAATCATTGCCAGACTTTGACAAGGACGGTGTGTGCAGAACAATGTGACGGCAGA

 221 CysTyrGlyProTyrValSerAspCysCysHisArgGluCysAlaGlyGlyCysSerGlyPro
 694 TGCTACGGACCTTACGTCAGTGACTGCTGCCATCGAGAATGTGCTGGAGGCTGCTCAGGACCT

 242 LysAspThrAspCysPheAlaCysMetAsnPheAsnAspSerGlyAlaCysValThrGlnCys
 757 AAGGACACAGACTGCTTTGCCTGCATGAATTTCAATGACAGTGGAGCATGTGTTACTCAGTGT

 263 ProGlnThrPheValTyrAsnProThrThrPheGlnLeuGluHisAsnPheAsnAlaLysTyr
 820 CCCCAAACCTTTGTCTACAATCCAACCACCTTTCAACTGGAGCACAATTTCAATGCAAAGTAC

 284 ThrTyrGlyAlaPheCysValLysLysCysProHisAsnPheValValAspSerSerSerCys
 883 ACATATGGAGCATTCTGTGTCAAGAAATGTCCACATAACTTTGTGGTAGATTCCAGTTCTTGT

 305 ValArgAlaCysProSerSerLysMetGluValGluGluAsnGlyIleLysMetCysLysPro
 946 GTGCGTGCCTGCCCTAGTTCCAAGATGGAAGTAGAAGAAAATGGGATTAAAATGTGTAAACCT

 326 CysThrAspIleCysProLysAlaCysAspGlyIleGlyThrGlySerLeuMetSerAlaGln
1009 TGCACTGACATTTGCCCAAAAGCTTGTGATGGCATTGGCACAGGATCATTGATGTCAGCTCAG

 347 ThrValAspSerSerAsnIleAspLysPheIleAsnCysThrLysIleAsnGlyAsnLeuIle
1072 ACTGTGGATTCCAGTAACATTGACAAATTCATAAACTGTACCAAGATCAATGGGAATTTGATC

 368 PheLeuValThrGlyIleHisGlyAspProTyrAsnAlaIleGluAlaIleAspProGluLys
1135 TTTCTAGTCACTGGTATTCATGGGGACCCTTACAATGCAATTGAAGCCATAGACCCAGAGAAA

 389 LeuAsnValPheArgThrValArgGluIleThrGlyPheLeuAsnIleGlnSerTrpProPro
1198 CTGAACGTCTTTCGGACAGTCAGAGAGATAACAGGTTTCCTGAACATACAGTCATGGCCACCA
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Figure 1/1

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 410 AsnMetThrAspPheSerValPheSerAsnLeuValThrIleProProAsnMetThrAspPhe
1261 AACATGACTGACTTCAGTGTTTTTTCTAACCTGGTGACCATTGGTGGAAGAGTACTCTATAGT

 431 SerValPheSerAsnLeuValThrIleGlnGlyIleThrSerLeuGlnPheGlnSerLeuLys
1324 GGCCTGTCCTTGCTTATCCTCAAGCAACAGGGCATCACCTCTCTACAGTTCCAGTCCCTGAAG

 452 GluIleSerAlaGlyAsnIleTyrIleThrAspAsnSerAsnLeuCysTyrTyrHisThrIle
1387 GAAATCAGCGCAGGAAACATCTATATTACTGACAACAGCAACCTGTGTTATTATCATACCATT

 473 AsnTrpThrThrLeuPheSerThrIleAsnGlnArgIleValIleArgAspAsnArgLysAla
1450 AACTGGACAACACTCTTCAGCACAATCAACCAGAGAATAGTAATCCGGGACAACAGAAAAGCT

 494 GluAsnCysThrAlaGluGlyMetValCysAsnHisLeuCysSerSerAspGlyCysTrpGly
1513 GAAAATTGTACTGCTGAAGGAATGGTGTGCAACCATCTGTGTTCCAGTGATGGCTGTTGGGGA

 515 ProGlyProAspGlnCysLeuSerCysArgArgPheSerArgGlyArgIleCysIleGluSer
1576 CCTGGGCCAGACCAATGTCTGTCGTGTCGCCGCTTCAGTAGAGGAAGGATCTGCATAGAGTCT

 536 CysAsnLeuTyrAspGlyGluPheArgGluPheGluAsnGlySerIleCysValGluCysAsp
1639 TGTAACCTCTATGATGGTGAATTTCGGGAGTTTGAGAATGGCTCCATCTGTGTGGAGTGTGAC

 557 ProGlnCysGluLysMetGluAspGlyLeuLeuThrCysHisGlyProGlyProAspAsnCys
1702 CCCCAGTGTGAGAAGATGGAAGATGGCCTCCTCACATGCCATGGACCGGGTCCTGACAACTGT

 578 ThrLysCysSerHisPheLysAspGlyProAsnCysValGluLysCysProAspGlyLeuGln
1765 ACAAAGTGCTCTCATTTTAAAGATGGCCCAAACTGTGTGGAAAAATGTCCAGATGGCTTACAG

 599 GlyAlaAsnSerPheIlePheLysTyrAlaAspProAspArgGluCysHisProCysHisPro
1828 GGGGCAAACAGTTTCATTTTCAAGTATGCTGATCCAGATCGGGAGTGCCACCCATGCCATCCA

 620 AsnCysThrGlnGlyCysAsnGlyProThrSerHisAspCysIleTyrTyrProTrpThrGly
1891 AACTGCACCCAAGGGTGTAACGGTCCCACTAGTCATGACTGCATTTACTACCCATGGACGGGC

 641 HisSerThrLeuProGlnHisAlaArgThrProLeuIleAlaAlaGlyValIleGlyGlyLeu
1954 CATTCCACTTTACCACAACATGCTAGAACTCCCCTGATTGCAGCTGGAGTAATTGGTGGGCTC

 662 PheIleLeuValIleValGlyLeuThrPheAlaValTyrValArgArgLysSerIleLysLys
2017 TTCATTCTGGTCATTGTGGGTCTGACATTTGCTGTTTATGTTAGAAGGAAGAGCATCAAAAAG

 683 LysArgAlaLeuArgArgPheLeuGluThrGluLeuValGluProLeuThrProSerGlyThr
2080 AAAAGAGCCTTGAGAAGATTCTTGGAAACAGAGTTGGTGGAACCATTAACTCCCAGTGGCACA

 704 AlaProAsnGlnAlaGlnLeuArgIleLeuLysGluThrGluLeuLysArgValLysValLeu
2143 GCACCCAATCAAGCTCAACTTCGTATTTTGAAAGAAACTGAGCTGAAGAGGGTAAAAGTCCTT

 725 GlySerGlyAlaPheGlyThrValTyrLysGlyIleTrpValProGluGlyGluThrValLys
2206 GGCTCAGGTGCTTTTGGAACGGTTTATAAAGGTATTTGGGTACCTGAAGGAGAAACTGTGAAG

 746 IleProValAlaIleLysIleLeuAsnGluThrThrGlyProLysAlaAsnValGluPheMet
2269 ATTCCTGTGGCTATTAAGATTCTTAATGAGACAACTGGTCCCAAGGCAAATGTGGAGTTCATG

 767 AspGluAlaLeuIleMetAlaSerMetAspHisProHisLeuValArgLeuLeuGlyValCys
2332 GATGAAGCTCTGATCATGGCAAGTATGGATCATCCACACCTAGTCCGGTTGCTGGGTGTGTGT

 788 LeuSerProThrIleGlnLeuValThrGlnLeuMetProHisGlyCysLeuLeuGluTyrVal
2395 CTGAGCCCAACCATCCAGCTGGTTACTCAACTTATGCCCCATGGCTGCCTGTTGGAGTATGTC
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Figure 1/2

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 809  HisGluHisLysAspAsnIleGlySerGlnLeuLeuLeuAsnTrpCysValGlnIleAlaLys
2458  CACGAGCACAAGGATAACATTGGATCACAACTGCTGCTTAACTGGTGTGTCCAGATAGCTAAG

 830  GlyMetMetTyrLeuGluGluArgArgLeuValHisArgAspLeuAlaAlaArgAsnValLeu
2521  GGAATGATGTACCTGGAAGAAAGACGACTCGTTCATCGGGATTTGGCAGCCCGTAATGTCTTA

 851  ValLysSerProAsnHisValLysIleThrAspPheGlyLeuAlaArgLeuLeuGluGlyAsp
2584  GTGAAATCTCCAAACCATGTGAAAATCACAGATTTTGGGCTAGCCAGACTCTTGGAAGGAGAT

 872  GluLysGluTyrAsnAlaAspGlyGlyLysMetProIleLysTrpMetAlaLeuGluCysIle
2647  GAAAAAGAGTACAATGCTGATGGAGGAAAGATGCCAATTAAATGGATGGCTCTGGAGTGTATA

 893  HisTyrArgLysPheThrHisGlnSerAspValTrpSerTyrGlyValThrIleTrpGluLeu
2710  CATTACAGGAAATTCACCCATCAGAGTGACGTTTGGAGCTATGGAGTTACTATATGGGAACTG

 914  MetThrPheGlyGlyLysProTyrAspGlyIleProThrArgGluIleProAspLeuLeuGlu
2773  ATGACCTTTGGAGGAAAACCCTATGATGGAATTCCAACGCGAGAAATCCCTGATTTATTAGAG

 935  LysGlyGluArgLeuProGlnProProIleCysThrIleAspValTyrMetValMetValLys
2836  AAAGGAGAACGTTTGCCTCAGCCTCCCATCTGCACTATTGACGTTTACATGGTCATGGTCAAA

 956  CysTrpMetIleAspAlaAspSerArgProLysPheLysGluLeuAlaAlaGluPheSerArg
2899  TGTTGGATGATTGATGCTGACAGTAGACCTAAATTTAAGGAACTGGCTGCTGAGTTTTCAAGG

 977  MetAlaArgAspProGlnArgTyrLeuValIleGlnGlyAspAspArgMetLysLeuProSer
2962  ATGGCTCGAGACCCTCAAAGATACCTAGTTATTCAGGGTGATGATCGTATGAAGCTTCCCAGT

 998  ProAsnAspSerLysPhePheGlnAsnLeuLeuAspGluGluAspLeuGluAspMetMetAsp
3025  CCAAATGACAGCAAGTTCTTTCAGAATCTCTTGGATGAAGAGGATTTGGAAGATATGATGGAT

1019  AlaGluGluTyrLeuValProGlnAlaPheAsnIleProProProIleTyrThrSerArgAla
3088  GCTGAGGAGTACTTGGTCCCTCAGGCTTTCAACATCCCACCTCCCATCTATACTTCCAGAGCA

1040  ArgIleAspSerAsnArgSerGluIleGlyHisSerProProProAlaTyrThrProMetSer
3151  AGAATTGACTCGAATAGGAGTGAAATTGGACACAGCCCTCCTCCTGCCTACACCCCCATGTCA

1061  GlyAsnGlnPheValTyrArgAspGlyGlyPheAlaAlaGluGlnGlyValSerValProTyr
3214  GGAAACCAGTTTGTATACCGAGATGGAGGTTTTGCTGCTGAACAAGGAGTGTCTGTGCCCTAC

1082  ArgAlaProThrSerThrIleProGluAlaProValAlaGlnGlyAlaThrAlaGluIlePhe
3277  AGAGCCCCAACTAGCACAATTCCAGAAGCTCCTGTGGCACAGGGTGCTACTGCTGAGATTTTT

1103  AspAspSerCysCysAsnGlyThrLeuArgLysProValAlaProHisValGlnGluAspSer
3340  GATGACTCCTGCTGTAATGGCACCCTACGCAAGCCAGTGGCACCCCATGTCCAAGAGGACAGT

1124  SerThrGlnArgTyrSerAlaAspProThrValPheAlaProGluArgSerProArgGlyGlu
3403  AGCACCCAGAGGTACAGTGCTGACCCCACCGTGTTTGCCCCAGAACGGAGCCCACGAGGAGAG

1145  LeuAspGluGluGlyTyrMetThrProMetArgAspLysProLysGlnGluTyrLeuAsnPro
3466  CTGGATGAGGAAGGTTACATGACTCCTATGCGAGACAAACCCAAACAAGAATACCTGAATCCA

1166  ValGluGluAsnProPheValSerArgArgLysAsnGlyAspLeuGlnAlaLeuAspAsnPro
3529  GTGGAGGAGAACCCTTTTGTTTCTCGGAGAAAAAATGGAGACCTTCAAGCATTGGATAATCCC

1187  GluTyrHisAsnAlaSerAsnGlyProProLysAlaGluAspGluTyrValAsnGluProLeu
3592  GAATATCACAATGCATCCAATGGTCCACCCAAGGCCGAGGATGAGTATGTGAATGAGCCACTG
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Figure 1/3

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1208 TyrLeuAsnThrPheAlaAsnThrLeuGlyLysAlaGluTyrLeuLysAsnAsnIleLeuSer
3655 TACCTCAACACCTTTGCCAACACCTTGGGAAAAGCTGAGTACCTGAAGAACAACATACTGTCA

1229 MetProGluLysAlaLysLysAlaPheAspAsnProAspTyrTrpAsnHisSerLeuProPro
3718 ATGCCAGAGAAGGCCAAGAAAGCGTTTGACAACCCTGACTACTGGAACCACAGCCTGCCACCT

1250 ArgSerThrLeuGlnHisProAspTyrLeuGlnGluTyrSerThrLysTyrPheTyrLysGln
3781 CGGAGCACCCTTCAGCACCCAGACTACCTGCAGGAGTACAGCACAAAATATTTTTATAAACAG

1271 AsnGlyArgIleArgProIleValAlaGluAsnProGluTyrLeuSerGluPheSerLeuLys
3844 AATGGGCGGATCCGGCCTATTGTGGCAGAGAATCCTGAATACCTCTCTGAGTTCTCCCTGAAG

1292 ProGlyThrValLeuProProProProTyrArgHisArgAsnThrValVal
3907 CCAGGCACTGTGCTGCCGCCTCCACCTTACAGACACCGGAATACTGTGGTGTAAGCTCAGTTG

3970 TGGTTTTTTAGGTGGAGAGACACACCTGCTCCAATTTCCCCACCCCCCTCTCTTTCTCTGGTG

4033 GTCTTCCTTCTACCCCAAGGCCAGTAGTTTTGACACTTCCCAGTGGAAGATACAGAGATGCAA

4096 TGATAGTTATGTGCTTACCTAACTTGAACATTAGAGGGAAAGACTGAAAGAGAAAGATAGGAG

4159 GAACCACAATGTTTCTTCATTTCTCTGCATGGGTTGGTCAGGAGAATGAAACAGCTAGAGAAG

4222 GACCAGAAAATGTAAGGCAATGCTGCCTACTATCAAACTAGCTGTCACTTTTTTTCTTTTTCT

4285 TTTTCTTTCTTTGTTTCTTTCTTCCTCTTCTTTTTTTTTTTTTTTTTAAAGCAGATGGTTGAA

4348 ACACCCATGCTATCTGTTCCTATCTGCAGGAACTGATGTGTGCATATTTAGCATCCCTGGAAA

4411 TCATAATAAAGTTTCCATTAGAACAAAAGAATAACATTTTCTATAACATATGATAGTGTCTGA

4474 AATTGAGAATCCAGTTTCTTTCCCCAGCAGTTTCTGTCCTAGCAAGTAAGAATGGCCAACTCA

4537 ACTTTCATAATTTAAAAATCTCCATTAAAGTTATAACTAGTAATTATGTTTTCAACACTTTTT

4600 GGTTTTTTTCATTTTGTTTTGCTCTGACCGATTCCTTTATATTTGCTCCCCTATTTTTGGCTT

4663 TAATTTCTAATTGCAAAGATGTTTACATCAAAGCTTCTTCACAGAATTTAAGCAAGAAATATT

4726 TTAATATAGTGAAATGGCCACTACTTTAAGTATACAATCTTTAAAATAAGAAAGGGAGGCTAA

4789 TATTTTTCATGCTATCAAATTATCTTCACCCTCATCCTTTACATTTTTCAACATTTTTTTTTC

4582 TCCATAAATGACACTACTTGATAGGCCGTTGGTTGTCTGAAGAGTAGAAGGGAAACTAAGAGA

4915 CAGTTCTCTGTGGTTCAGGAAAACTACTGATACTTTCAGGGGTGGCCCAATGAGGGAATCCAT

4978 TGAACTGGAAGAAACACACTGGATTGGGTATGTCTACCTGGCAGATACTCAGAAATGTAGTTT

5041 GCACTTAAGCTGTAATTTTATTTGTTCTTTTTCTGAACTCCATTTTGGATTTTGAATCAAGCA

5104 ATATGGAAGCAACCAGCAAATTAACTAATTTAAGTACATTTTTAAAAAAAGAGCTAAGATAAA

5167 GACTGTGGAAATGCCAAACCAAGCAAATTAGGAACCTTGCAACGGTATCCAGGGACTATGATG

5230 AGAGGCCAGCACATTATCTTCATATGTCACCTTTGCTACGCAAGGAAATTTGTTCAGTTCGTA
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Figure 1/4

5293 TACTTCGTAAGAAGGAATGCGAGTAAGGATTGGCTTGAATTCCATGGAATTTCTAGTATGAGA

5356 CTATTTATATGAAGTAGAAGGTAACTCTTTGCACATAAATTGGTATAATAAAAAGAAAAACAC

5419 AAACATTCAAAGCTTAGGGATAGGTCCTTGGGTCAAAAGTTGTAAATAAATGTGAAACATCTT

5482 CTCAAAAAAAAAAAAAAAAA

Figure 1/5

```
   1                                                MetLysProAlaThrGlyLeuTrpValTrp
   1 AATTGTCAGCACGGGATCTGAGACTTCCAAAAAATGAAGCCGGCGACAGGACTTTGGGTCTGG

  11 ValSerLeuLeuValAlaAlaGlyThrValGlnProSerAspSerGlnSerValCysAlaGly
  64 GTGAGCCTTCTCGTGGCGGCGGGGACCGTCCAGCCCAGCGATTCTCAGTCAGTGTGTGCAGGA

  32 ThrGluAsnLysLeuSerSerLeuSerAspLeuGluGlnGlnTyrArgAlaLeuArgLysTyr
 127 ACGGAGAATAAACTGAGCTCTCTCTCTGACCTGGAACAGCAGTACCGAGCCTTGCGCAAGTAC

  53 TyrGluAsnCysGluValValMetGlyAsnLeuGluIleThrSerIleGluHisAsnArgAsp
 190 TATGAAAACTGTGAGGTTGTCATGGGCAACCTGGAGATAACCAGCATTGAGCACAACCGGGAC

  74 LeuSerPheLeuArgSerValArgGluValThrGlyTyrValLeuValAlaLeuAsnGlnPhe
 253 CTCTCCTTCCTGCGGTCTGTTCGAGAAGTCACAGGCTACGTGTTAGTGGCTCTTAATCAGTTT

  95 ArgTyrLeuProLeuGluAsnLeuArgIleIleArgGlyThrLysLeuTyrGluAspArgTyr
 316 CGTTACCTGCCTCTGGAGAATTTACGCATTATTCGTGGGACAAAACTTTATGAGGATCGATAT

 116 AlaLeuAlaIlePheLeuAsnTyrArgLysAspGlyAsnPheGlyLeuGlnGluLeuGlyLeu
 379 GCCTTGGCAATATTTTTAAACTACAGAAAAGATGGAAACTTTGGACTTCAAGAACTTGGATTA

 137 LysAsnLeuThrGluIleLeuAsnGlyGlyValTyrValAspGlnAsnLysPheLeuCysTyr
 442 AAGAACTTGACAGAAATCCTAAATGGTGGAGTCTATGTAGACCAGAACAAATTCCTTTGTTAT

 158 AlaAspThrIleHisTrpGlnAspIleValArgAsnProTrpProSerAsnLeuThrLeuVal
 505 GCAGACACCATTCATTGGCAAGATATTGTTCGGAACCCATGGCCTTCCAACTTGACTCTTGTG

 179 SerThrAsnGlySerSerGlyCysGlyArgCysHisLysSerCysThrGlyArgCysTrpGly
 568 TCAACAAATGGTAGTTCAGGATGTGGACGTTGCCATAAGTCCTGTACTGGCCGTTGCTGGGGA

 200 ProThrGluAsnHisCysGlnThrLeuThrArgThrValCysAlaGluGlnCysAspGlyArg
 631 CCCACAGAAAATCATTGCCAGACTTTGACAAGGACGGTGTGTGCAGAACAATGTGACGGCAGA

 221 CysTyrGlyProTyrValSerAspCysCysHisArgGluCysAlaGlyGlyCysSerGlyPro
 694 TGCTACGGACCTTACGTCAGTGACTGCTGCCATCGAGAATGTGCTGGAGGCTGCTCAGGACCT

 242 LysAspThrAspCysPheAlaCysMetAsnPheAsnAspSerGlyAlaCysValThrGlnCys
 757 AAGGACACAGACTGCTTTGCCTGCATGAATTTCAATGACAGTGGAGCATGTGTTACTCAGTGT

 263 ProGlnThrPheValTyrAsnProThrThrPheGlnLeuGluHisAsnPheAsnAlaLysTyr
 820 CCCCAAACCTTTGTCTACAATCCAACCACCTTTCAACTGGAGCACAATTTCAATGCAAAGTAC

 284 ThrTyrGlyAlaPheCysValLysLysCysProHisAsnPheValValAspSerSerSerCys
 883 ACATATGGAGCATTCTGTGTCAAGAAATGTCCACATAACTTTGTGGTAGATTCCAGTTCTTGT

 305 ValArgAlaCysProSerSerLysMetGluValGluGluAsnGlyIleLysMetCysLysPro
 946 GTGCGTGCCTGCCCTAGTTCCAAGATGGAAGTAGAAGAAAATGGGATTAAAATGTGTAAACCT

 326 CysThrAspIleCysProLysAlaCysAspGlyIleGlyThrGlySerLeuMetSerAlaGln
1009 TGCACTGACATTTGCCCAAAAGCTTGTGATGGCATTGGCACAGGATCATTGATGTCAGCTCAG

 347 ThrValAspSerSerAsnIleAspLysPheIleAsnCysThrLysIleAsnGlyAsnLeuIle
1072 ACTGTGGATTCCAGTAACATTGACAAATTCATAAACTGTACCAAGATCAATGGGAATTTGATC

 368 PheLeuValThrGlyIleHisGlyAspProTyrAsnAlaIleGluAlaIleAspProGluLys
1135 TTTCTAGTCACTGGTATTCATGGGGACCCTTACAATGCAATTGAAGCCATAGACCCAGAGAAA

 389 LeuAsnValPheArgThrValArgGluIleThrGlyPheLeuAsnIleGlnSerTrpProPro
1198 CTGAACGTCTTTCGGACAGTCAGAGAGATAACAGGTTTCCTGAACATACAGTCATGGCCACCA
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Figure 2/1

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 410 AsnMetThrAspPheSerValPheSerAsnLeuValThrIleProProAsnMetThrAspPhe
1261 AACATGACTGACTTCAGTGTTTTTTCTAACCTGGTGACCATTGGTGGAAGAGTACTCTATAGT

 431 SerValPheSerAsnLeuValThrIleGlnGlyIleThrSerLeuGlnPheGlnSerLeuLys
1324 GGCCTGTCCTTGCTTATCCTCAAGCAACAGGGCATCACCTCTCTACAGTTCCAGTCCCTGAAG

 452 GluIleSerAlaGlyAsnIleTyrIleThrAspAsnSerAsnLeuCysTyrTyrHisThrIle
1387 GAAATCAGCGCAGGAAACATCTATATTACTGACAACAGCAACCTGTGTTATTATCATACCATT

 473 AsnTrpThrThrLeuPheSerThrIleAsnGlnArgIleValIleArgAspAsnArgLysAla
1450 AACTGGACAACACTCTTCAGCACAATCAACCAGAGAATAGTAATCCGGGACAACAGAAAAGCT

 494 GluAsnCysThrAlaGluGlyMetValCysAsnHisLeuCysSerSerAspGlyCysTrpGly
1513 GAAAATTGTACTGCTGAAGGAATGGTGTGCAACCATCTGTGTTCCAGTGATGGCTGTTGGGGA

 515 ProGlyProAspGlnCysLeuSerCysArgArgPheSerArgGlyArgIleCysIleGluSer
1576 CCTGGGCCAGACCAATGTCTGTCGTGTCGCCGCTTCAGTAGAGGAAGGATCTGCATAGAGTCT

 536 CysAsnLeuTyrAspGlyGluPheArgGluPheGluAsnGlySerIleCysValGluCysAsp
1639 TGTAACCTCTATGATGGTGAATTTCGGGAGTTTGAGAATGGCTCCATCTGTGTGGAGTGTGAC

 557 ProGlnCysGluLysMetGluAspGlyLeuLeuThrCysHisGlyProGlyProAspAsnCys
1702 CCCCAGTGTGAGAAGATGGAAGATGGCCTCCTCACATGCCATGGACCGGGTCCTGACAACTGT

 578 ThrLysCysSerHisPheLysAspGlyProAsnCysValGluLysCysProAspGlyLeuGln
1765 ACAAAGTGCTCTCATTTTAAAGATGGCCCAAACTGTGTGGAAAAATGTCCAGATGGCTTACAG

 599 GlyAlaAsnSerPheIlePheLysTyrAlaAspProAspArgGluCysHisProCysHisPro
1828 GGGGCAAACAGTTTCATTTTCAAGTATGCTGATCCAGATCGGGAGTGCCACCCATGCCATCCA

 620 AsnCysThrGlnGlyCysAsnGlyProThrSerHisAspCysIleTyrTyrProTrpThrGly
1891 AACTGCACCCAAGGGTGTAACGGTCCCACTAGTCATGACTGCATTTACTACCCATGGACGGGC

 641 HisSerThrLeuProGlnHisAlaArgThrProLeuIleAlaAlaGlyValIleGlyGlyLeu
1954 CATTCCACTTTACCACAACATGCTAGAACTCCCCTGATTGCAGCTGGAGTAATTGGTGGGCTC

 662 PheIleLeuValIleValGlyLeuThrPheAlaValTyrValArgArgLysSerIleLysLys
2017 TTCATTCTGGTCATTGTGGGTCTGACATTTGCTGTTTATGTTAGAAGGAAGAGCATCAAAAAG

 683 LysArgAlaLeuArgArgPheLeuGluThrGluLeuValGluProLeuThrProSerGlyThr
2080 AAAAGAGCCTTGAGAAGATTCTTGGAAACAGAGTTGGTGGAACCATTAACTCCCAGTGGCACA

 704 AlaProAsnGlnAlaGlnLeuArgIleLeuLysGluThrGluLeuLysArgValLysValLeu
2143 GCACCCAATCAAGCTCAACTTCGTATTTTGAAAGAAACTGAGCTGAAGAGGGTAAAAGTCCTT

 725 GlySerGlyAlaPheGlyThrValTyrLysGlyIleTrpValProGluGlyGluThrValLys
2206 GGCTCAGGTGCTTTTGGAACGGTTTATAAAGGTATTTGGGTACCTGAAGGAGAAACTGTGAAG

 746 IleProValAlaIleLysIleLeuAsnGluThrThrGlyProLysAlaAsnValGluPheMet
2269 ATTCCTGTGGCTATTAAGATTCTTAATGAGACAACTGGTCCCAAGGCAAATGTGGAGTTCATG

 767 AspGluAlaLeuIleMetAlaSerMetAspHisProHisLeuValArgLeuLeuGlyValCys
2332 GATGAAGCTCTGATCATGGCAAGTATGGATCATCCACACCTAGTCCGGTTGCTGGGTGTGTGT

 788 LeuSerProThrIleGlnLeuValThrGlnLeuMetProHisGlyCysLeuLeuGluTyrVal
2395 CTGAGCCCAACCATCCAGCTGGTTACTCAACTTATGCCCCATGGCTGCCTGTTGGAGTATGTC
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Figure 2/2

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 809 HisGluHisLysAspAsnIleGlySerGlnLeuLeuLeuAsnTrpCysValGlnIleAlaLys
2458 CACGAGCACAAGGATAACATTGGATCACAACTGCTGCTTAACTGGTGTGTCCAGATAGCTAAG

 830 GlyMetMetTyrLeuGluGluArgArgLeuValHisArgAspLeuAlaAlaArgAsnValLeu
2521 GGAATGATGTACCTGGAAGAAAGACGACTCGTTCATCGGGATTTGGCAGCCCGTAATGTCTTA

 851 ValLysSerProAsnHisValLysIleThrAspPheGlyLeuAlaArgLeuLeuGluGlyAsp
2584 GTGAAATCTCCAAACCATGTGAAAATCACAGATTTTGGGCTAGCCAGACTCTTGGAAGGAGAT

 872 GluLysGluTyrAsnAlaAspGlyGlyLysMetProIleLysTrpMetAlaLeuGluCysIle
2647 GAAAAAGAGTACAATGCTGATGGAGGAAAGATGCCAATTAAATGGATGGCTCTGGAGTGTATA

 893 HisTyrArgLysPheThrHisGlnSerAspValTrpSerTyrGlyValThrIleTrpGluLeu
2710 CATTACAGGAAATTCACCCATCAGAGTGACGTTTGGAGCTATGGAGTTACTATATGGGAACTG

 914 MetThrPheGlyGlyLysProTyrAspGlyIleProThrArgGluIleProAspLeuLeuGlu
2773 ATGACCTTTGGAGGAAAACCCTATGATGGAATTCCAACGCGAGAAATCCCTGATTTATTAGAG

 935 LysGlyGluArgLeuProGlnProProIleCysThrIleAspValTyrMetValMetValLys
2836 AAAGGAGAACGTTTGCCTCAGCCTCCCATCTGCACTATTGACGTTTACATGGTCATGGTCAAA

 956 CysTrpMetIleAspAlaAspSerArgProLysPheLysGluLeuAlaAlaGluPheSerArg
2899 TGTTGGATGATTGATGCTGACAGTAGACCTAAATTTAAGGAACTGGCTGCTGAGTTTTCAAGG

 977 MetAlaArgAspProGlnArgTyrLeuValIleGlnGlyAspAspArgMetLysLeuProSer
2962 ATGGCTCGAGACCCTCAAAGATACCTAGTTATTCAGGGTGATGATCGTATGAAGCTTCCCAGT

 998 ProAsnAspSerLysPhePheGlnAsnLeuLeuAspGluGluAspLeuGluAspMetMetAsp
3025 CCAAATGACAGCAAGTTCTTTCAGAATCTCTTGGATGAAGAGGATTTGGAAGATATGATGGAT

1019 AlaGluGluTyrLeuValProGlnAlaPheAsnIleProProProIleTyrThrSerArgAla
3088 GCTGAGGAGTACTTGGTCCCTCAGGCTTTCAACATCCCACCTCCCATCTATACTTCCAGAGCA

1040 ArgIleAspSerAsnArgSerValArgAsnAsnTyrIleHisIleSerTyrSerPhe***
3151 AGAATTGACTCGAATAGGAGTGTAAGAAATAATTATATACACATATCATATTCTTTCTGA

3211 GATATAAAATCATGTAATAGTTCATAAGCACTAACATTTCAAAATAATTATATAGCTCAAATC

3274 AATGTGATGCCTAGATTAAAAATATACCATACCCACAAAAGATGTGCCAATCTTGCTATATGT

3337 AGTTAATTTTGGAAGACAAGCATGGACAATACAACATGTACTCTGAAATACCTTCAAGATTTC

3400 AGAAGCAAAACATTTTCCTCATCTTAATTTATTTAAAACAAATCTTAACTTTAAAAAACAATT

3463 CCAACTAATAAAACCATTATGTGTATATAAATAAATGAAAATTCCTACCAAGTAGGCTTTCTA

3526 CTTTTCTTTCTTAAAAAGATATTATGATATATTAGTCAAGAAGTAATACAAGTATAAATCTCT

3589 TTCACTTATTTAAGAAAAATTAAATATTTTCTGTCAAGTTGAAGTAGAAACACAGAAAACCGT

3652 GCAGTCCTTTGAACCTAATCACATCGAAAAGGCTGCTGAGAAGTAGATTTTTGTTTTTAAGAA

3715 GTAGATTTAAGTTTTGAAGGAAGTTTCTGAAAACACTTTACATTTTAAATGTTAAACCTACTC

3778 TATATGAATTCCATTCTTTCTTTGAAAGCTGTCAAATCCATGCATTTATTTTTATAAATTCAT

3841 TCCTCATACATTCAACATATATTGAGTACCACTGTATGTGAAGCATTAGTATACATTTAAGAC
```

Figure 2/3

3904 TCAAAGAATTTTGATACAACTTCTGCTTTCAAGAAGTGAAAACCTTAATCAAAGAATCATACA

3967 GATAGAGGGACTGCATAGTAAGTGCTGTAATCCAGTATTCACTGACCAGTACGGAGCATGAAG

4030 AAGTAGTAAATTTGTGTCTGTAATCAGTTTCTTCCATTGATAAGATATAAACATGATGCTTAA

4093 TTTTTTCTAGAAGATAATTCTTTTCTCTTAATCTAAGAACATTATCATAGCTAGTAGAACCGA

4156 CAGCATCCGATTTCTCTTGACCATAGCCATAAGAATATCTTCAACTTGCTGCTCATTATCTAA

4219 CAAACATAATTTTCTTTATTTCATATTGATTGTAATAAGTAATATCCCCCTGGAAGTTTACTA

4282 TTCAACACATATATGTTAACCTCCTTAATTCCTTAAACAAACTTCATGAGGTTCTATTATTAT

4345 CATCCCCTTCTTTCAAAGGAAGAAACTTGCCACAGAGAAGTCAGGTGATATGACTGGTGTCAC

4408 ACAGCTAGTCAGTGGAAGAGAGGAATAAGTAATCTAGATATCTGCCTACTACACTGTAGGTTT

4471 GCTTCAAAGTTACTGAAGYCATGTTATTTCCATGATGTGATTAGAGTCTGGGACTTGTCTTGT

4534 TTGGGAAATTTCCCAGGTGGTTTTCTTATAAAATGCATCTCAAATCTGCTCTACACCTTTTAC

4597 TCATCTACCTCCATTTAGAAGATCTGATATGGAAAGAGACAAAGATGGAGACCTCAATTATTT

4660 TTTCTTTTCTGTTAAAAATATTATAGTACAACTGAAACTTATCACATGCCAATGGGGAATAGA

4723 TAACTAAAAGTTTAAAATTAGATCAATGGATAGGTAAATGAATAATCNTTCTTTTGCTTGTGA

4786 GAGGGGAAGGAAAAGCGGTTAAGGTGGTATAAAGGAGGCTCCTCTGTACACTTGCAAAATGAT

4849 CAAATTATATACCCTTGTATTTATAATTTTAAGTGACAAATTCATTACTTCTGGTTACAACAG

4912 TGAAATTTAAAAAAAAAATAGTTTTTCTTTCTTAGCTTGCAATGCTATAAATCTTTTTCTTTTT

4975 ATAAGAATTCTTACATTTCAGCTTTTTGTTCATTTTAATTTATAATTCTCAGTGCAAGAAATT

5038 CTTAATAAAGGTTTGAGCTAGCTAGATGGAATTATTGAGACAAAGTCTAAATCACCCGTGGAC

5101 TTATTTGACCTTTAGCCATCATTTCTTATTCCACATTATAAAACAATGTTACCTGTAGATTTC

5164 TTTTTACTTTTTCAGTCCTTGGAAAAGAAATGGTGATTAAATATCATTATATCATTTTATGTT

5227 CAGGCATTTAAAAAGCTTTATTTGTCATCTATATTGTCCTAATAGTTTTCAGTCTGGCTTTAC

5290 GTAACTTTTACGGAAATTTCTAACATGTACAAATGCCATGTTCCTCCTTTCTTTCCTACATGG

5353 CTGAATTAGAAAACAAATTACTTCCATTTTAAGTTTGGCTAAATTAGAAAACAAATTACTACC

5416 ATTTTAAGTTTGGTGGCTAAATAACGTGCTAAGGGAACATCTTAAAAAGTGAATTTTGATCAA

5479 ATATTTCTTAAGCATATGTGATAGACTTTGAAACCAAAAAAAAAAAAAAAAAAAAAAAAAAA

5542 AAAAAAAAAAAAAA

Figure 2/4

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   1 CATTAGCTGCAATTGATCAAGTGACTGAGAGAAGGGCAACATTCCATGCAACAGTATAG

  60 TGGTATGGAAAGCCCTGGATGTTGAAATCTAGCTTCAAAAAGCCTGTCTGGAAATGTAGT

   1                                                       MetAlaSerMet
 120 TAATTGGATGAAGTGAGAAGAGATAAAACCAGAGAGGAAGCTCTGATCATGGCAAGTATG

   5 AspHisProHisLeuValArgLeuLeuGlyValCysLeuSerProThrIleGlnLeuVal
 180 GATCATCCACACCTAGTCCGGTTGCTGGGTGTGTGTCTGAGCCCAACCATCCAGCTGGTT

  25 ThrGlnLeuMetProHisGlyCysLeuLeuGluTyrValHisGluHisLysAspAsnIle
 240 ACTCAACTTATGCCCCATGGCTGCCTGTTGGAGTATGTCCACGAGCACAAGGATAACATT

  45 GlySerGlnLeuLeuLeuAsnTrpCysValGlnIleAlaLysGlyMetMetTyrLeuGlu
 300 GGATCACAACTGCTGCTTAACTGGTGTGTCCAGATAGCTAAGGGAATGATGTACCTGGAA

  65 GluArgArgLeuValHisArgAspLeuAlaAlaArgAsnValLeuValLysSerProAsn
 360 GAAAGACGACTCGTTCATCGGGATTTGGCAGCCCGTAATGTCTTAGTGAAATCTCCAAAC

  85 HisValLysIleThrAspPheGlyLeuAlaArgLeuLeuGluGlyAspGluLysGluTyr
 420 CATGTGAAAATCACAGATTTTGGGCTAGCCAGACTCTTGGAAGGAGATGAAAAAGAGTAC

 105 AsnAlaAspGlyGlyLysMetProIleLysTrpMetAlaLeuGluCysIleHisTyrArg
 480 AATGCTGATGGAGGAAAGATGCCAATTAAATGGATGGCTCTGGAGTGTATACATTACAGG

 125 LysPheThrHisGlnSerAspValTrpSerTyrGlyValThrIleTrpGluLeuMetThr
 540 AAATTCACCCATCAGAGTGACGTTTGGAGCTATGGAGTTACTATATGGGAACTGATGACC

 145 PheGlyGlyLysProTyrAspGlyIleProThrArgGluIleProAspLeuLeuGluLys
 600 TTTGGAGGAAAACCCTATGATGGAATTCCAACGCGAGAAATCCCTGATTTATTAGAGAAA

 165 GlyGluArgLeuProGlnProProIleCysThrIleAspValTyrMetValMetValLys
 660 GGAGAACGTTTGCCTCAGCCTCCCATCTGCACTATTGACGTTTACATGGTCATGGTCAAA

 185 CysTrpMetIleAspAlaAspSerArgProLysPheLysGluLeuAlaAlaGluPheSer
 720 TGTTGGATGATTGATGCTGACAGTAGACCTAAATTTAAGGAACTGGCTGCTGAGTTTTCA

 205 ArgMetAlaArgAspProGlnArgTyrLeuValIleGlnGlyAspAspArgMetLysLeu
 780 AGGATGGCTCGAGACCCTCAAAGATACCTAGTTATTCAGGGTGATGATCGTATGAAGCTT

 225 ProSerProAsnAspSerLysPhePheGlnAsnLeuLeuAspGluGluAspLeuGluAsp
 840 CCCAGTCCAAATGACAGCAAGTTCTTTCAGAATCTCTTGGATGAAGAGGATTTGGAAGAT

 245 MetMetAspAlaGluGluTyrLeuValProGlnAlaPheAsnIleProProProIleTyr
 900 ATGATGGATGCTGAGGAGTACTTGGTCCCTCAGGCTTTCAACATCCCACCTCCCATCTAT

 265 ThrSerArgAlaArgIleAspSerAsnArgSerGluIleGlyHisSerProProProAla
 960 ACTTCCAGAGCAAGAATTGACTCGAATAGGAGTGAAATTGGACACAGCCCTCCTCCTGCC

 285 TyrThrProMetSerGlyAsnGlnPheValTyrArgAspGlyGlyPheAlaAlaGluGln
1020 TACACCCCCATGTCAGGAAACCAGTTTGTATACCGAGATGGAGGTTTTGCTGCTGAACAA

 305 GlyValSerValProTyrArgAlaProThrSerThrIleProGluAlaProValAlaGln
1080 GGAGTGTCTGTGCCCTACAGAGCCCCAACTAGCACAATTCCAGAAGCTCCTGTGGCACAG

 325 GlyAlaThrAlaGluIlePheAspAspSerCysCysAsnGlyThrLeuArgLysProVal
1140 GGTGCTACTGCTGAGATTTTTGATGACTCCTGCTGTAATGGCACCCTACGCAAGCCAGTG
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Figure 3/1

```
 345 AlaProHisValGlnGluAspSerSerThrGlnArgTyrSerAlaAspProThrValPhe
1200 GCACCCCATGTCCAAGAGGACAGTAGCACCCAGAGGTACAGTGCTGACCCCACCGTGTTT

 365 AlaProGluArgSerProArgGlyGluLeuAspGluGluGlyTyrMetThrProMetArg
1260 GCCCCAGAACGGAGCCCACGAGGAGAGCTGGATGAGGAAGGTTACATGACTCCTATGCGA

 385 AspLysProLysGlnGluTyrLeuAsnProValGluGluAsnProPheValSerArgArg
1320 GACAAACCCAAACAAGAATACCTGAATCCAGTGGAGGAGAACCCTTTTGTTTCTCGGAGA

 405 LysAsnGlyAspLeuGlnAlaLeuAspAsnProGluTyrHisAsnAlaSerAsnGlyPro
1380 AAAAATGGAGACCTTCAAGCATTGGATAATCCCGAATATCACAATGCATCCAATGGTCCA

 425 ProLysAlaGluAspGluTyrValAsnGluProLeuTyrLeuAsnThrPheAlaAsnThr
1440 CCCAAGGCCGAGGATGAGTATGTGAATGAGCCACTGTACCTCAACACCTTTGCCAACACC

 445 LeuGlyLysAlaGluTyrLeuLysAsnAsnIleLeuSerMetProGluLysAlaLysLys
1500 TTGGGAAAAGCTGAGTACCTGAAGAACAACATACTGTCAATGCCAGAGAAGGCCAAGAAA

 465 AlaPheAspAsnProAspTyrTrpAsnHisSerLeuProProArgSerThrLeuGlnHis
1560 GCGTTTGACAACCCTGACTACTGGAACCACAGCCTGCCACCTCGGAGCACCCTTCAGCAC

 485 ProAspTyrLeuGlnGluTyrSerThrLysTyrPheTyrLysGlnAsnGlyArgIleArg
1620 CCAGACTACCTGCAGGAGTACAGCACAAAATATTTTTATAAACAGAATGGGCGGATCCGG

 505 ProIleValAlaGluAsnProGluTyrLeuSerGluPheSerLeuLysProGlyThrVal
1680 CCTATTGTGGCAGAGAATCCTGAATACCTCTCTGAGTTCTCCCTGAAGCCAGGCACTGTG

 525 LeuProProProProTyrVal
1740 CTGCCGCCTCCACCTTACAGACACCGGAATACTGTGGTGTAAGCTCAGTTGTGGTTTTTT

1800 AGGTGGAGAGACACACCTGCTCCAATTTCCCCACCCCCCTCTCTTTCTCTGGTGGTCTTC

1860 CTTCTACCCCCAGTAGTTTTGACACTTCCCAGTGGAAGATACAGAGATGCAATGATAGTT

1920 ATGTGCTTACCTAACTTGAACATTAGAGGGAAAGACTGAAAGAGAAAGATAGGAGGAACC

1980 ACAATGTTTCTTCATTTCTCTGCATGGGTTGGTCAGGAGAATGAAACAGCTAGAGAAGGA

2040 CCAGAAAATGTAAGGCAATGCTGCCTACTATCAAACTAGCTGTCACTTTTTTTCTTTTTC

2100 TTTTTCTTTCTTTGTTTCTTTCTTCCTCTTCTTTTTTTTTTTTTTTTTAAAGCAGATGGT

2160 TGAAACACCCATGCTATCTGTTCCTATCTGCAGGAACTGATGTGTGCATATTTAGCATCC

2220 CTGGAAATCATAATAAAGTTTCCATTAGAACAAAAGAATAACATTTTCTATAACATATGA

2280 TAGTGTCTGAAATTGAGAATCCAGTTTCTTTCCCCAGCAGTTTCTGTCCTAGCAAGTAAG

2340 AATGGCCAACTCAACTTTCATAATTTAAAAATCTCCATTAAAGTTATAACTAGTAATTAT

2400 GTTTTCAACACTTTTTGGTTTTTTTCATTTTGTTTTGCTCTGACCGATTCCTTTATATTT

2460 GCTCCCCTATTTTTGGCTTTAATTTCTAATTGCAAAGATGTTTACATCAAAGCTTCTTCA

2520 CAGAATTTAAGCAAGAAATATTTTAATATAGTGAAATGGCCACTACTTTAAGTATACAAT

2580 CTTTAAAATAAGAAAGGGAGGCTAATATTTTTCATGCTATCAAATTATCTTCACCCTCAT
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Figure 3/2

2640 CCTTTACATTTTTCAACATTTTTTTTTCTCCATAAATGACACTACTTGATAGGCCGTTGG

2700 TTGTCTGAAGAGTAGAAGGGAAACTAAGAGACAGTTCTCTGTGGTTCAGGAAAACTACTG

2760 ATACTTTCAGGGGTGGCCCAATGAGGGAATCCATTGAACTGGAAGAAACACACTGGATTG

2820 GGTATGTCTACCTGGCAGATACTCAGAAATGTAGTTTGCACTTAAGCTGTAATTTTATTT

2880 GTTCTTTTTCTGAACTCCATTTTGGATTTTGAATCAAGCAATATGGAAGCAACCAGCAAA

2940 TTAACTAATTTAAGTACATTTTTAAAAAAAGAGCTAAGATAAAGACTGTGGAAATGCCAA

3000 ACCAAGCAAATTAGGAACCTTGCAACGGTATCCAGGGACTATGATGAGAGGCCAGCACAT

3060 TATCTTCATATGTCACCTTTGCTACGCAAGGAAATTTGTTCAGTTCGTATACTTCGTAAG

3120 AAGGAATGCGAGTAAGGATTGGCTTGAATTCCATGGAATTTCTAGTATGAGACTATTTAT

3180 ATGAAGTAGAAGGTAACTCTTTGCACATAAATTGGTATAATAAAAAGAAAAACACAAACA

3240 TTCAAAGCTTAGGGATAGGTCCTTGGGTCAAAAGTTGTAAATAAATGTGAAACATCTTCT

3300 CAAAAAAAAAAAAAAAA

Figure 3/3

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MKPATGLWVWVSLLVAAGTVQPSDSQSVCAGTENKLSSLSDLEQQYRALRKYYENCEVVM  60
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
MKPATGLWVWVSLLVAAGTVQPSDSQSVCAGTENKLSSLSDLEQQYRALRKYYENCEVVM  60

GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAIF 120
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAIF 120

LNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLTLVST 180
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
LNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLTLVST 180

NGSSGCGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRECAGGCSG 240
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
NGSSGCGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRECAGGCSG 240

PKDTDCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTYGAFCVKKCPHNFVVD 300
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PKDTDCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTYGAFCVKKCPHNFVVD 300

SSSCVRACPSSKMEVEENGIKMCKPCTDICPKACDGIGTGSLMSAQTVDSSNIDKFINCT 360
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
SSSCVRACPSSKMEVEENGIKMCKPCTDICPKACDGIGTGSLMSAQTVDSSNIDKFINCT 360

KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL 420
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL 420

VTIGGRVLYSGLSLLILKQQGITSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFST 480
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VTIGGRVLYSGLSLLILKQQGITSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFST 480

INQRIVIRDNRKAENCTAEGMVCNHLCSSDGCWGPGPDQCLSCRRFSRGRICIESCNLYD 540
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
INQRIVIRDNRKAENCTAEGMVCNHLCSSDGCWGPGPDQCLSCRRFSRGRICIESCNLYD 540

GEFREFENGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHFKDGPNCVEKCPDGLQGA 600
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GEFREFENGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHFKDGPNCVEKCPDGLQGA 600

NSFIFKYADPDRECHPCHPNCTQGCNGPTSHDCIYYPWTGHSTLPQHARTPLIAAGVIGG 660
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
NSFIFKYADPDRECHPCHPNCTQGCNGPTSHDCIYYPWTGHSTLPQHARTPLIAAGVIGG 660

LFILVIVGLTFAVYVRRKSIKKKRALRRFLETELVEPLTPSGTAPNQAQLRILKETELKR 720
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
LFILVIVGLTFAVYVRRKSIKKKRALRRFLETELVEPLTPSGTAPNQAQLRILKETELKR 720

VKVLGSGAFGTVYKGIWVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASMDHPHL 780
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VKVLGSGAFGTVYKGIWVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASMDHPHL 780
```

Figure 4/1

```
VRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLV   840
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLV   840

HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFTHQ   900
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFTHQ   900

SDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMID   960
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
SDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMID   960

ADSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLDEEDLEDMMDAE  1020
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
ADSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLDEEDLEDMMDAE  1020

EYLVPQAFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQGVSVP  1080
:::::::::::::::::::::::::::          :.
EYLVPQAFNIPPPIYTSRARIDSNRVRNNYIHIS-YSF                         1057

YRAPTSTIPEAPVAQGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQRYSADPTVFAPERS  1140
PRGELDEEGYMTPMRDKPKQEYLNPVEENPFVSRRKNGDLQALDNPEYHNASNGPPKAED  1200
EYVNEPLYLNTFANTLGKAEYLKNNILSMPEKAKKAFDNPDYWNHSLPPRSTLQHPDYLQ  1260
EYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGTVLPPPPYRHRNTVV              1308
```

Figure 4/2

```
MKPATGLWVWVSLLVAAGTVQPSDSQSVCAGTENKLSSLSDLEQQYRALRKYYENCEVVM     60
GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAIF    120
LNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLTLVST    180
NGSSGCGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRECAGGCSG    240
PKDTDCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTYGAFCVKKCPHNFVVD    300
SSSCVRACPSSKMEVEENGIKMCKPCTDICPKACDGIGTGSLMSAQTVDSSNIDKFINCT    360
KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL    420
VTIGGRVLYSGLSLLILKQQGITSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFST    480
INQRIVIRDNRKAENCTAEGMVCNHLCSSDGCWGPGPDQCLSCRRFSRGRICIESCNLYD    540
GEFREFENGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHFKDGPNCVEKCPDGLQGA    600
NSFIFKYADPDRECHPCHPNCTQGCNGPTSHDCIYYPWTGHSTLPQHARTPLIAAGVIGG    660

VKVLGSGAFGTVYKGIWVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASMDHPHL    780
                                                :::::::::::::
                                                EALIMASMDHPHL     13

VRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLV    840
:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLV     73

HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFTHQ    900
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPIKWMALECIHYRKFTHQ    133

SDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMID    960
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
SDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMID    193

ADSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLDEEDLEDMMDAE   1020
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
ADSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLDEEDLEDMMDAE    253

EYLVPQAFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQGVSVP   1080
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EYLVPQAFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQGVSVP    313

YRAPTSTIPEAPVAQGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQRYSADPTVFAPERS   1140
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
YRAPTSTIPEAPVAQGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQRYSADPTVFAPERS    373

PRGELDEEGYMTPMRDKPKQEYLNPVEENPFVSRRKNGDLQALDNPEYHNASNGPPKAED   1200
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PRGELDEEGYMTPMRDKPKQEYLNPVEENPFVSRRKNGDLQALDNPEYHNASNGPPKAED    433

EYVNEPLYLNTFANTLGKAEYLKNNILSMPEKAKKAFDNPDYWNHSLPPRSTLQHPDYLQ   1260
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EYVNEPLYLNTFANTLGKAEYLKNNILSMPEKAKKAFDNPDYWNHSLPPRSTLQHPDYLQ    493

EYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGTVLPPPPYRHRNTVV               1308
::::::::::::::::::::::::::::::::::::::::::::::::
EYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGTVLPPPPYRHRNTVV                541
```

Figure 5

TK
HER4 680 IK-KKRALRRFL-ETELVEPLTPSGTAPNQAQLR
EGFR 649 .V-R..T...L.Q.R.....E.....L..
HER2 680 Q.IR.YTH..L.Q.....AM.....M.
HER3 651 RIQN...H..Y.ERG.SI...D..-EKA.KVLA.
HER4 778 PHLVRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWCVQIAKGMMYLEERRLVHRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNA
EGFR 748 .VC....I..TS.V..I.....F....D..R.....Y.....D.....T.Q.....K..GAE....H.
HER2 780 .YVS....I..TS.V.....Y....DH.R.NRGRL...D.....M.....S...DV.....DI..T..H.
HER3 750 A.I.....L.PGSSL.....YL.L.S..DH.RQ.RGAL.P.....GV.....Y....HGM...N.....L...SQ.QVA...V.D..PP.D.QLLY
HER4 878 DGGKMPIKWMALECIHYRKFTHQSDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQPPICTIDVYMVMVKCWMIDADSRPKFKELAAEFSRM
EGFR 848 E...V.....S.LH.IY.....V.....S.....AS..SSI.....I.....R..II...K.
HER2 880 ....V.....S.LR.R.....V.....A.....I.....SEC..R.R..VS.....
HER3 850 SEA.T.....S..FG.Y.....V.....AE..A.LRLA.V.....A..Q.....ENIR.T.....N..T..
HER4 978 ARDPQRYLVIQGDDRMKL-PSPNDSKFFQNLLDEEDLEDMMDAEEYLVPQ-AFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQ
EGFR 948 .....E..H.-...T..N.YRA.M....MD.VV..D....I..QG.FSS.S--...----------
HER2 980 .....FV...NE.-LGP-A..L..T.YRS..EDD.MG.LV.....QG.FC.D.APGAGGMVHHRHRSSSTRSGGGDL----------TL
HER3 950 ....P.....KRESGPGIA.G.EPHGLTNKK.E.VE..PEL.LDLD.EAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPMNQGNLGGSCQE
HER4 1076 GVSVPYRAPTSTIPEAPVA--QGATAEIFDDSCCNGTLRKPVAPHVQEDSSTQRYSADPTVFAPERSPRGELDEEGYMTPMRDKPKQEYLNPVEENPFYS
EGFR 1008 ----------T.LLSSLS..SN--NSTVACIDRNGLQSCPIK....FL....S...GALT.D.I-----DDTFL------.VP..I.QS-----.P
HER2 1062 ---GLEPSEEA.RS.L.PSE..QSDV..GDLGM.AAKGLQSLPTHDP.PL....E.....PL.S-------ETD..VA.LTCS.QP..V.QPDVR.QPP
HER3 1050 SAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSHCRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHLKG
HER4 1174 RRKNGDLQALDNPEYHNASNGPPKAEDEYVNEPLYLNTFANTLGKAEYLK-----NNILSMPEKAKKAFDNPDYWNHSLPPRSTLQHPDYLQEYSTKYFY
EGFR 1075 K.PA.SV---Q...V....QPLN.APS-----RD.H.QDPHSTAV.NP...NT--VQPTCVNSTFDSP.-----H.AQKGSHQISLDN...Q.DFFP.EA-
HER2 1151 SPRE.P.P.ARPAGATLERAKTLSPGKNG.VKDVF--A.GGAVENP...TPQGGAAPQPHP.PAFSP....LY..DQDP.E.QAPPST----------
HER3 1150 TPSREGTLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPIMPTAGTTPDEDYEYMNRQRD
HER4 1269 KQNGRIRPI-VAENPEYLSEFSLKPGTVLPPPPYRHRNTVV 1308
EGFR 1158 .P..IFKGS-T....A...RVAPQSSEFIGA 1186
HER2 1237 ----FKGTPT.....GLDVPV 1255
HER3 1250 GGGPGGDYAAMGACPASEQGYEEMRAFQGPGHQAPHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT 1323

Figure 6/1

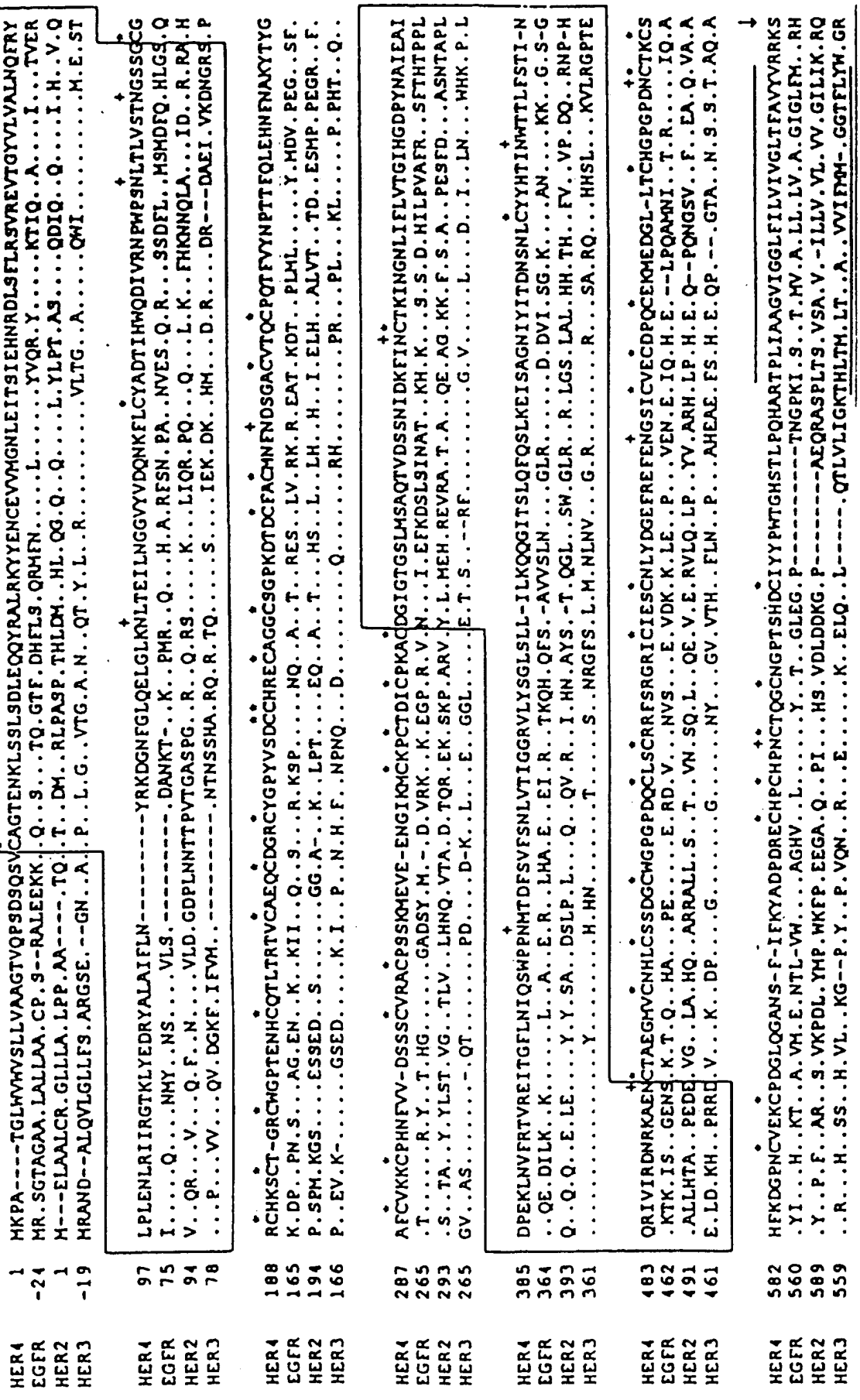


Figure 6/2

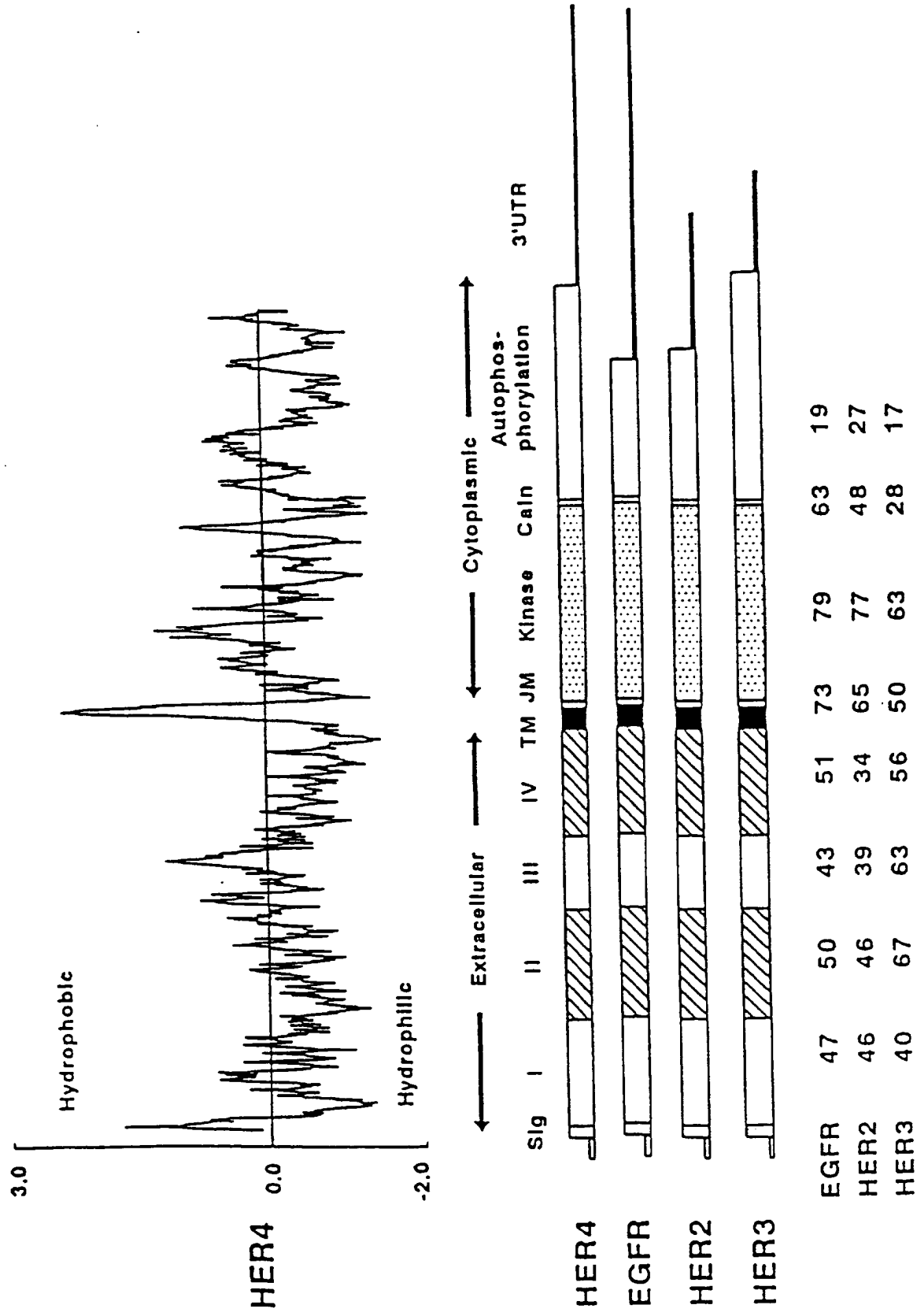

HER4
3.0
0.0
-2.0
Hydrophobic
Hydrophilic
Extracellular
Cytoplasmic
Autophos-
phorylation
3'UTR
Sig I II III IV TM JM Kinase Caln
HER4 47 50 43 51 73 79 63 19
EGFR 46 46 39 34 65 77 48 27
HER2 46 67 63 56 50 63 28 17
HER3 40

Figure 7

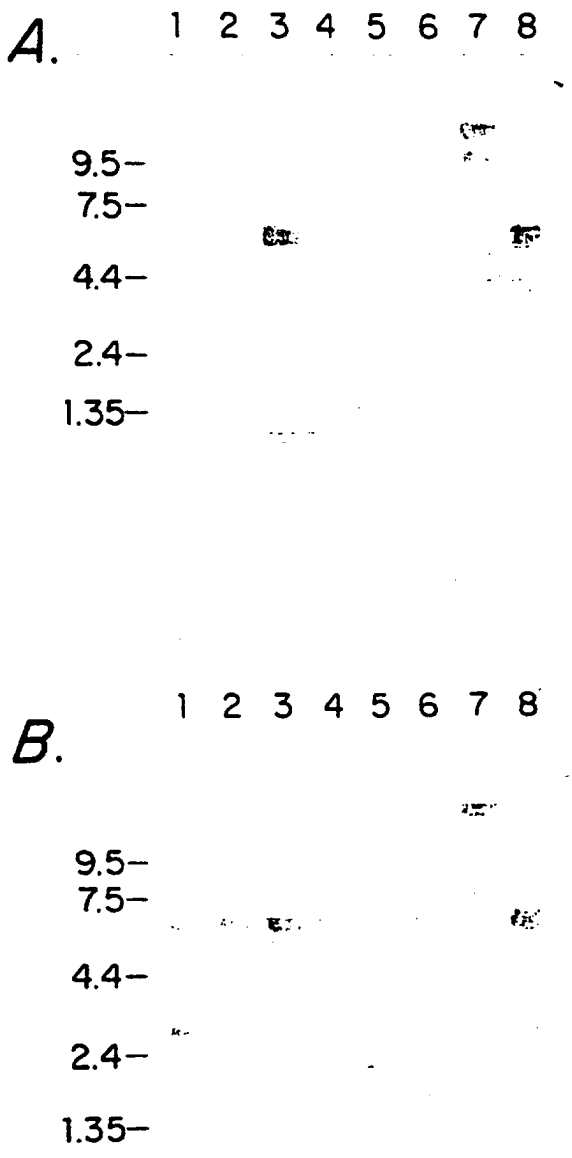


Figure 8

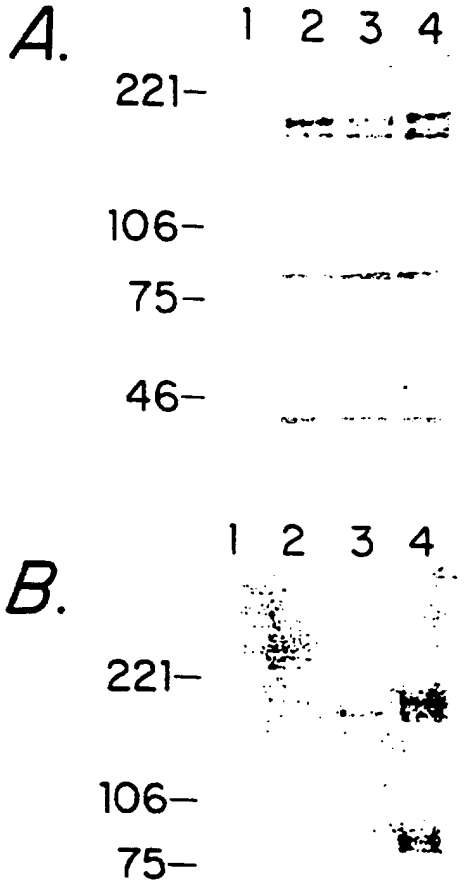


Figure 9

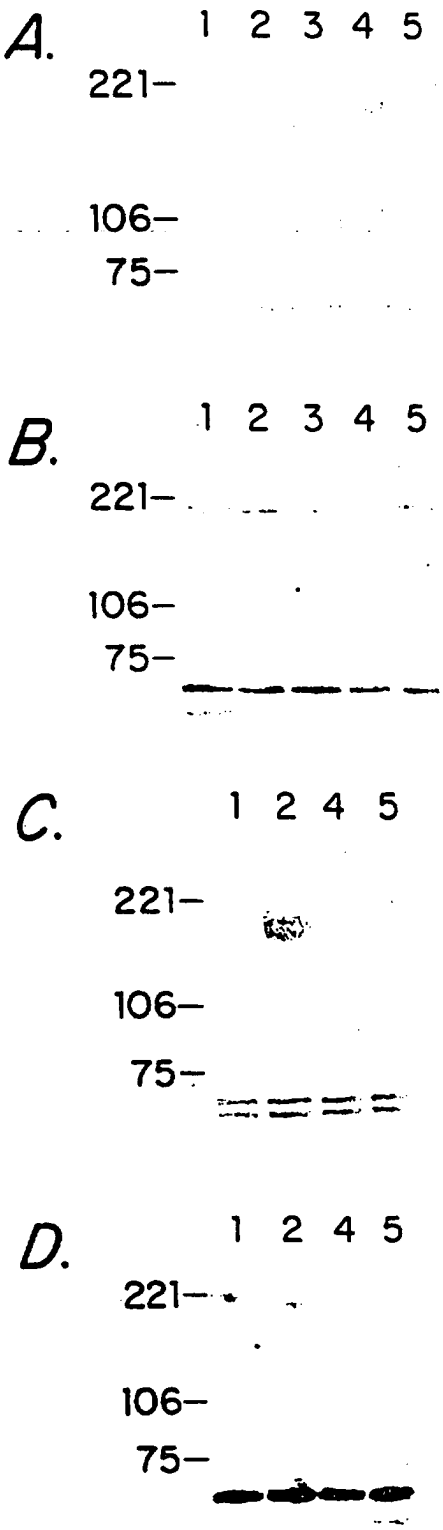

A.
1 2 3 4 5
221–
106–
75–
B.
1 2 3 4 5
221–
106–
75–
C.
1 2 4 5
221–
106–
75–
D.
1 2 4 5
221–
106–
75–

Figure 10

Fig. 11A

Fig. 11B

Fig. 11C

Fig. 11D

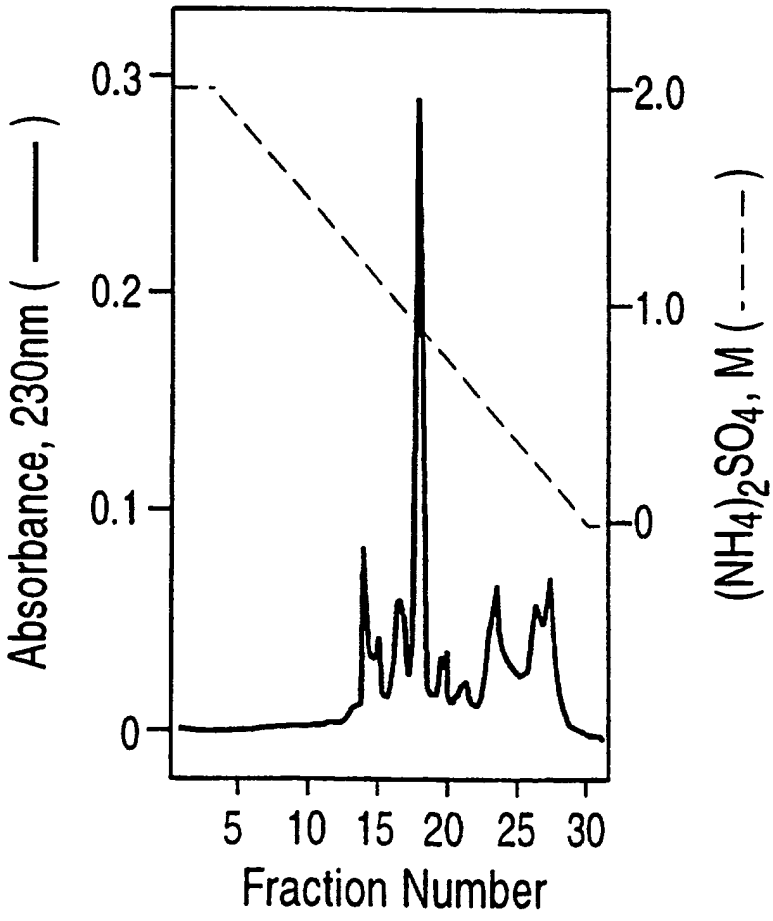


Fig. 11E

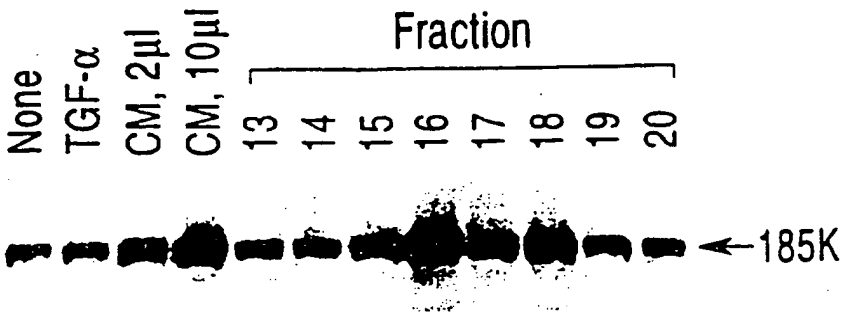
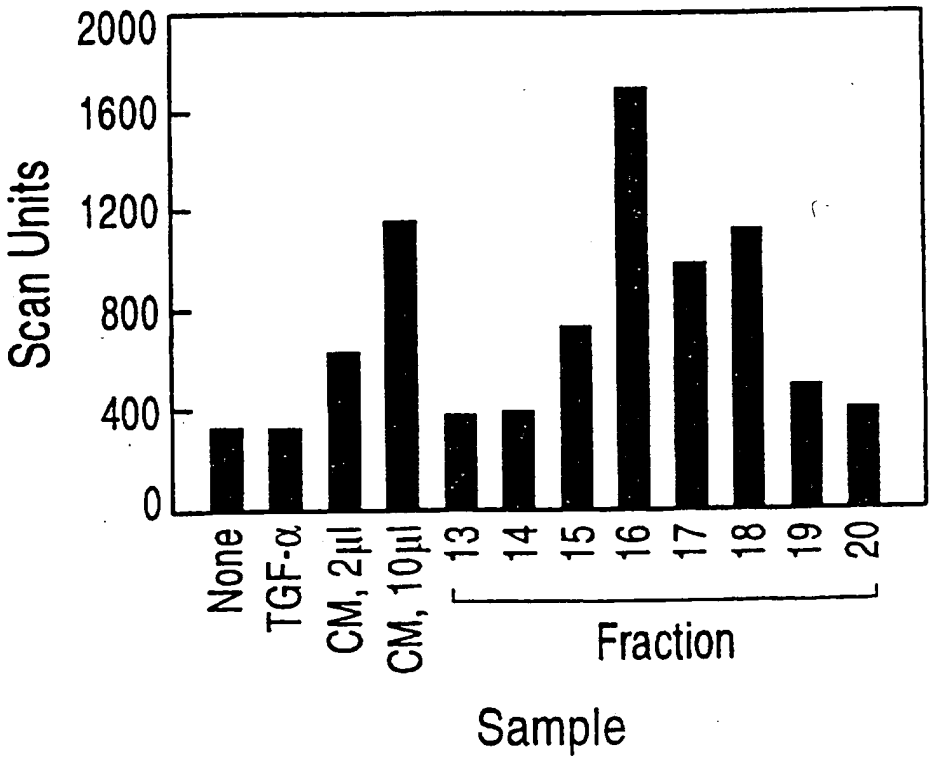


Fig. 11F



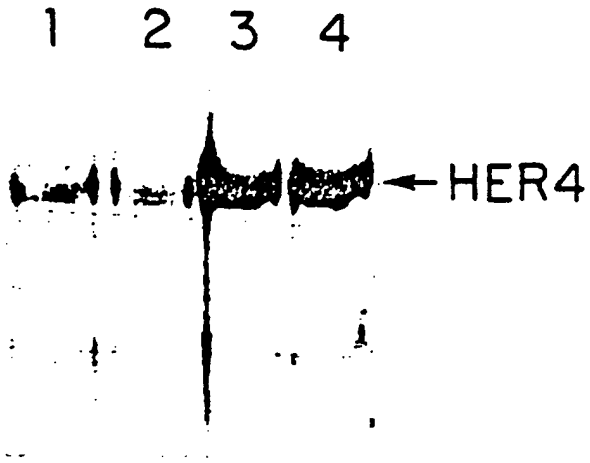


Figure 12A

Figure 12B

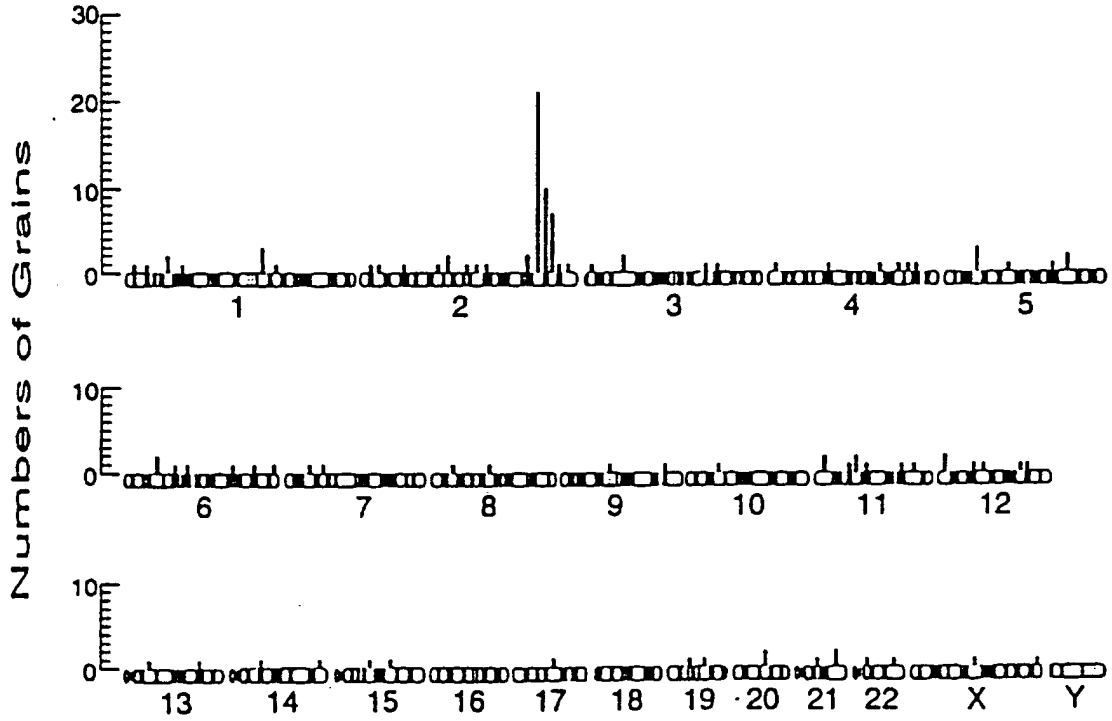

Numbers of Grains
30
20
10
0
1
2
3
4
5
10
0
6
7
8
9
10
11
12
10
0
13
14
15
16
17
18
19
20
21
22
X
Y

Figure 13A

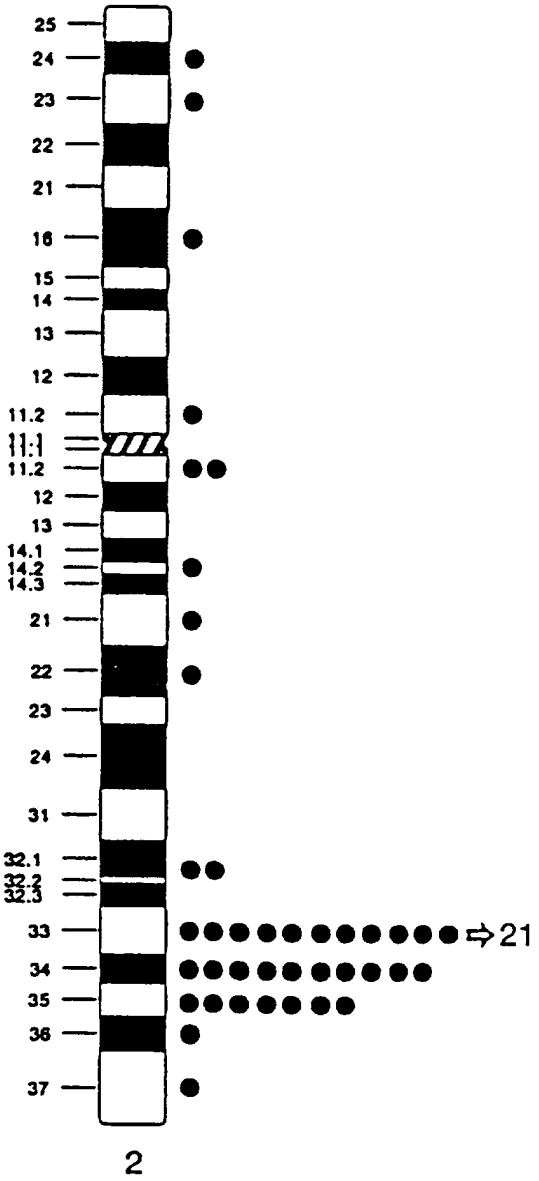

25
24
23
22
21
16
15
14
13
12
11.2
11.1
11.2
12
13
14.1
14.2
14.3
21
22
23
24
31
32.1
32.2
32.3
33
34
35
36
37
⇨21
2

Figure 13B

MKPATGLWVWVSLLVAAGTVQPSDSQSVCAGTENKLSSLSDLEQQYRALRKYYENCEVVM
GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAIF
LNYRKDGNFGLQELGLKNLTEILNGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLTLVST
NGSSGCGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRECAGGCSG
PKDTDCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTYGAFCVKKCPHNFVVD
SSSCVRACPSSKMEVEENGIKMCKPCTDICPKACDGIGTGSLMSAQTVDSSNIDKFINCT
KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL
VTIGGRVLYSGLSLLILKQQGITSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFST
INQRIVIRDNRKAENCTAEGMVCNHLCSSDGCWGPGPDQCLSCRRFSRGRICIESCNLYD
GEFREFENGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHFKDGPNCVEKCPDGLQGA
NSFIFKYADPDRECHPCHPNCTQGCNGPTSHDCIYYPWTGHSTLPQDPVKVKALEGFPRL
VGPDFFGCAEPANTFLDPEEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHVAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

Bold = Signal Sequence

Figure 14

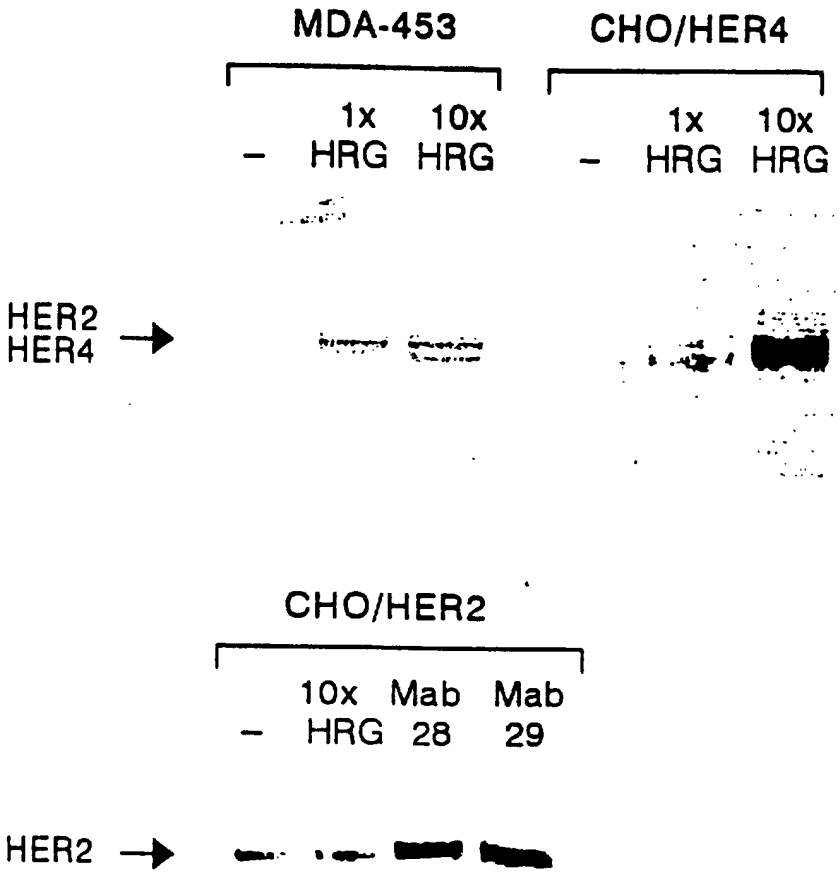

MDA-453
CHO/HER4
1x
10x
–
HRG
HRG
1x
10x
–
HRG
HRG
HER2
HER4
CHO/HER2
10x
Mab
Mab
–
HRG
28
29
HER2

Figure 15

Fig. 16A

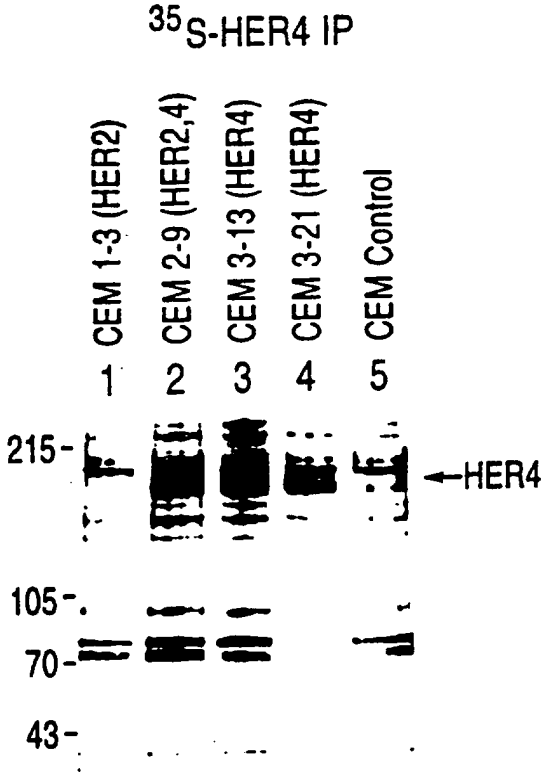


Fig. 16B

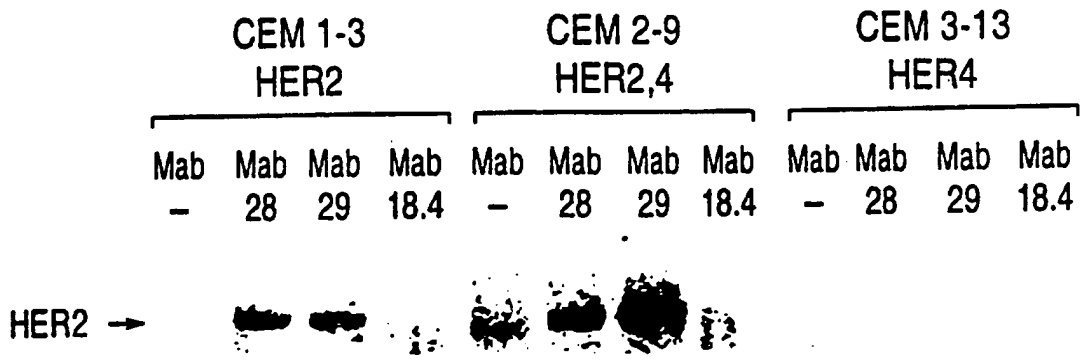


Fig. 16C

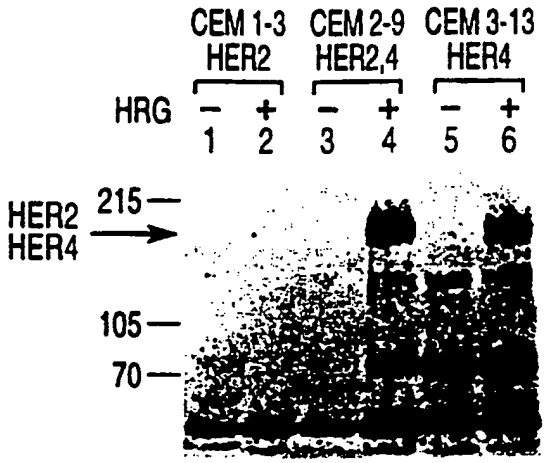


Figure 17A

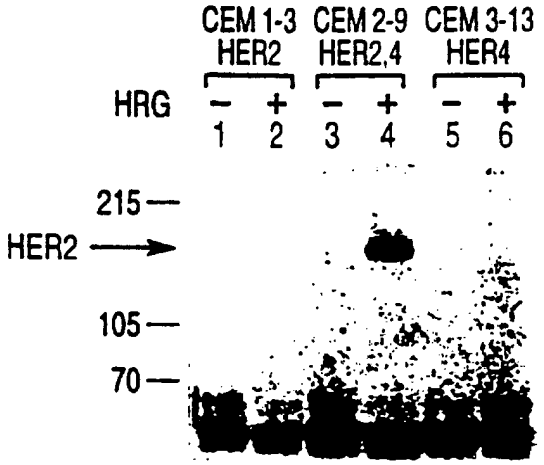


Figure 17B

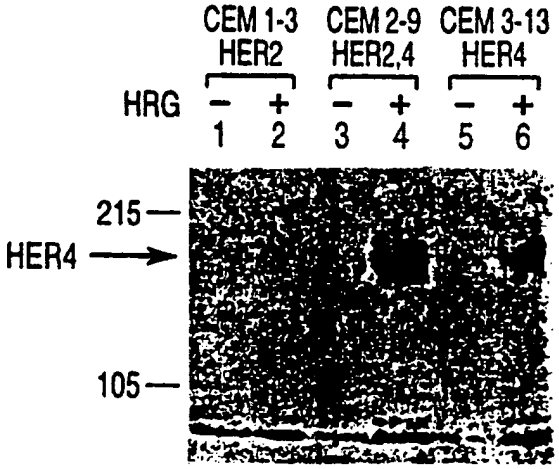


Figure 17C

Figure 18

Fig. 19A

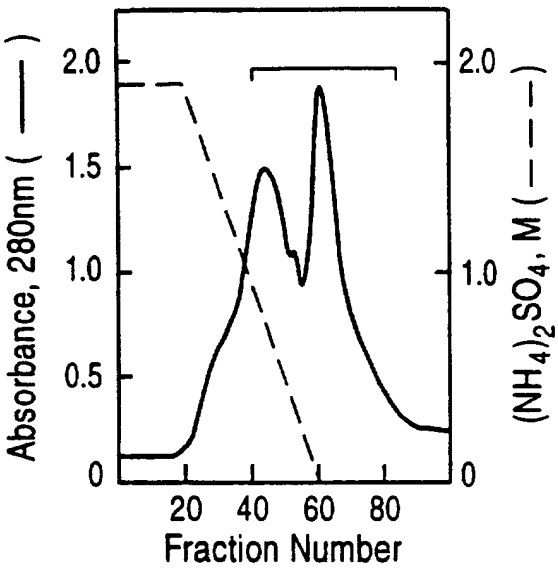


Fig. 19C

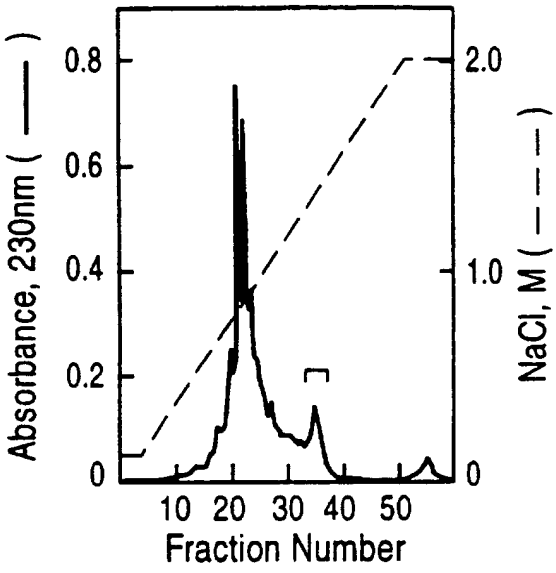


Fig. 19B

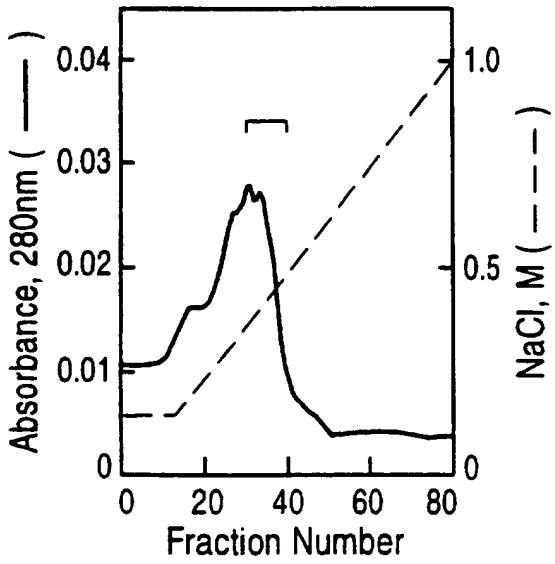
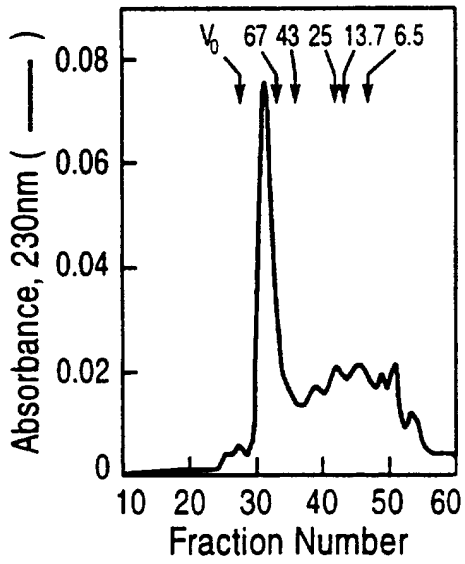


Fig. 19D



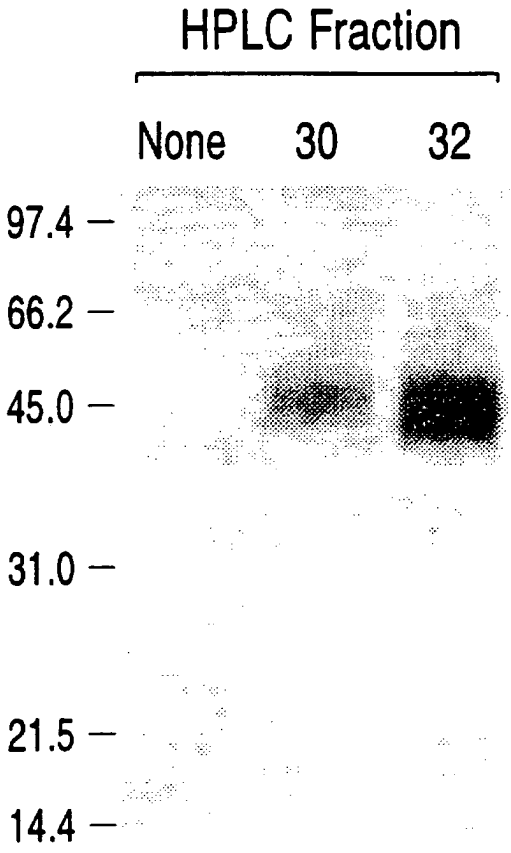


Figure 20

Fig. 21A

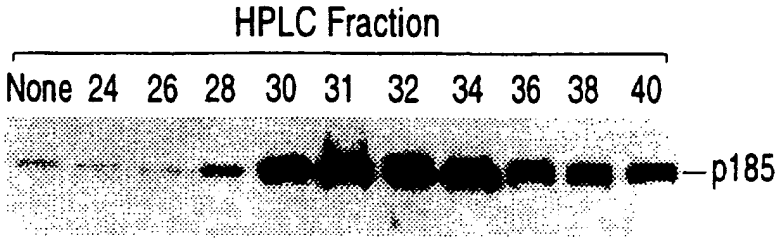


Fig. 21B

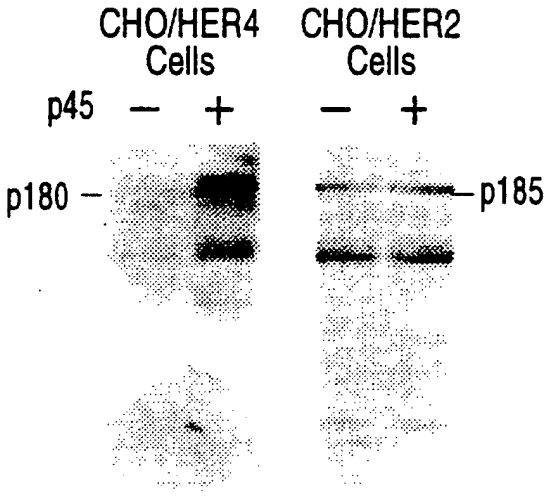


Fig. 21C

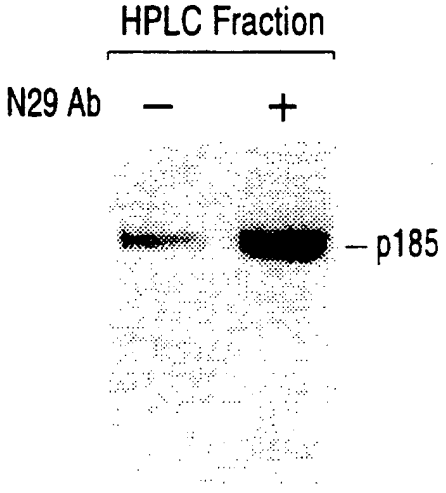


Fig. 22A

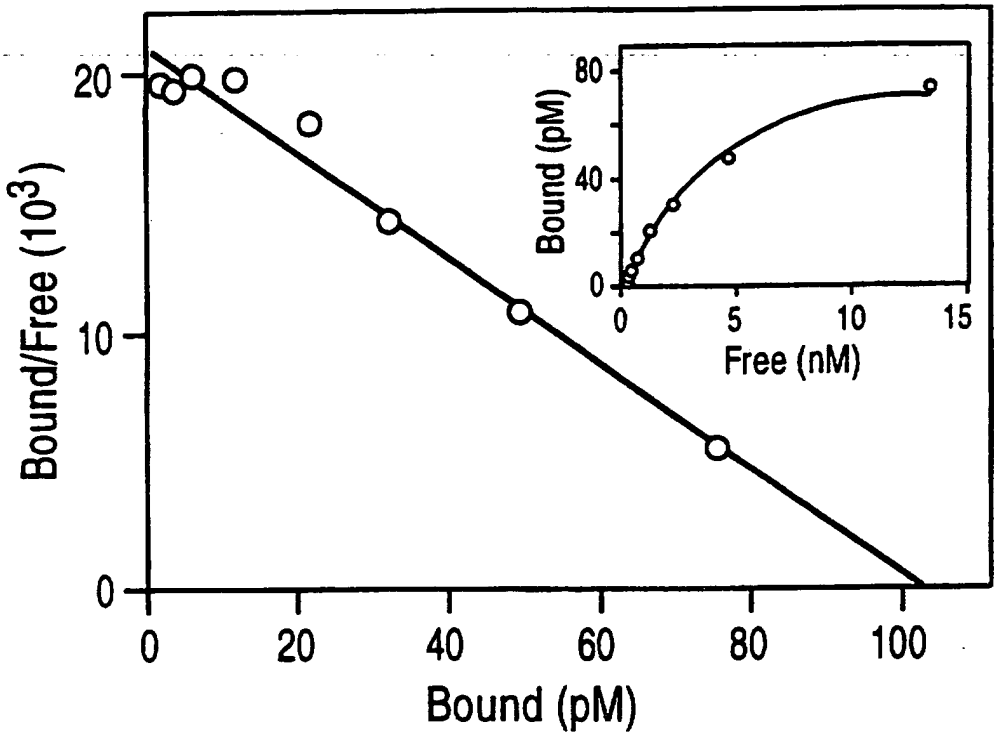
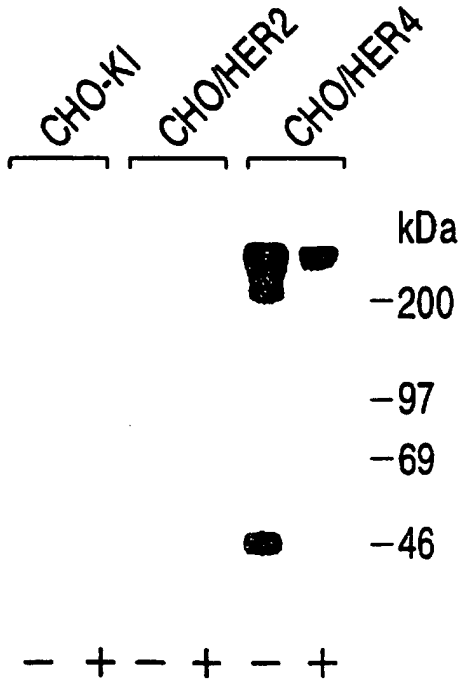


Fig. 22B



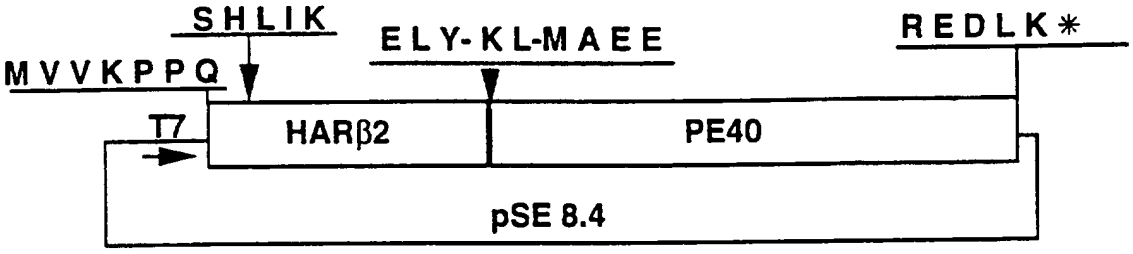


Figure 23A

AR leader **MVVKPPQNKTESENTSDKPKRKKKGGKNGKNRRNR--**

HRGβ2 **SHLIKCAEKEKTFCVNGGECFTVKDLSNPSRYLCKC**

PNEFTGDRCQNYVMASFYKAEELY

Figure 23B

```
ATG GTA GTT AAG CCC CCC CAA AAC AAG ACG GAA AGT GAA AAT ACT TCA      48
Met Val Val Lys Pro Pro Gln Asn Lys Thr Glu Ser Glu Asn Thr Ser
 1               5                  10                  15

GAT AAA CCC AAA AGA AAG AAA AAG GGA GGC AAA AAT GGA AAA AAT AGA      96
Asp Lys Pro Lys Arg Lys Lys Lys Gly Gly Lys Asn Gly Lys Asn Arg
            20                  25                  30

AGA AAC AGA AGC CAT CTC ATA AAG TGT GCG GAG AAG GAG AAA ACT TTC     144
Arg Asn Arg Ser His Leu Ile Lys Cys Ala Glu Lys Glu Lys Thr Phe
        35                  40                  45

TGT GTG AAT GGG GGC GAG TGC TTC ACG GTG AAG GAC CTG TCA AAC CCG     192
Cys Val Asn Gly Gly Glu Cys Phe Thr Val Lys Asp Leu Ser Asn Pro
    50                  55                  60

TCA AGA TAC TTG TGC AAG TGC CCG AAC GAA TTT ACT GGC GAC CGT TGC     240
Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys
65                  70                  75                  80

CAG AAC TAT GTT ATG GCA TCT TTT TAC AAA GCG GAG GAA CTC TAC AAG     288
Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr Lys
                85                  90                  95

CTT ATG GCC GAG GAA GGC GGC AGC CTG GCC GCG CTG ACC GCG CAC CAG     336
Leu Met Ala Glu Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
            100                 105                 110

GCT TGC CAC CTG CCG CTG GAG ACT TTC ACC CGT CAT CGC CAG CCG CGC     384
Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        115                 120                 125

GGC TGG GAA CAA CTG GAG CAG TGC GGC TAT CCG GTG CAG CGG CTG GTC     432
Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    130                 135                 140

GCC CTC TAC CTG GCG GCG CGG CTG TCG TGG AAC CAG GTC GAC CAG GTG     480
Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
145                 150                 155                 160

ATC CGC AAC GCC CTG GCC AGC CCC GGC AGC GGC GGC GAC CTG GGC GAA     528
Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                165                 170                 175

GCG ATC CGC GAG CAG CCG GAG CAG GCC CGT CTG GCC CTG ACC CTG GCC     576
Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            180                 185                 190

GCC GCC GAG AGC GAG CGC TTC GTC CGG CAG GGC ACC GGC AAC GAC GAG     624
Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        195                 200                 205

GCC GGC GCG GCC AAC GCC GAC GTG GTG AGC CTG ACC TGC CCG GTC GCC     672
Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    210                 215                 220

GCC GGT GAA TGC GCG GGC CCG GCG GAC AGC GGC GAC GCC CTG CTG GAG     720
Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
225                 230                 235                 240
```

Figure 24A

```
CGC AAC TAT CCC ACT GGC GCG GAG TTC CTC GGC GAC GGC GGC GAC GTC      768
Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                245                 250                 255

AGC TTC AGC ACC CGC GGC ACG CAG AAC TGG ACG GTG GAG CGG CTG CTC      816
Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            260                 265                 270

CAG GCG CAC CGC CAA CTG GAG GAG CGC GGC TAT GTG TTC GTC GGC TAC      864
Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        275                 280                 285

CAC GGC ACC TTC CTC GAA GCG GCG CAA AGC ATC GTC TTC GGC GGG GTG      912
His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    290                 295                 300

CGC GCG CGC AGC CAG GAC CTC GAC GCG ATC TGG CGC GGT TTC TAT ATC      960
Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
305                 310                 315                 320

GCC GGC GAT CCG GCG CTG GCC TAC GGC TAC GCC CAG GAC CAG GAA CCC     1008
Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
                325                 330                 335

GAC GCA CGC GGC CGG ATC CGC AAC GGT GCC CTG CTG CGG GTC TAT GTG     1056
Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
            340                 345                 350

CCG CGC TCG AGC CTG CCG GGC TTC TAC CGC ACC AGC CTG ACC CTG GCC     1104
Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        355                 360                 365

GGC GGC GAG GCG GCG GGC GAG GTC GAA CGG CTG ATC GGC CAT CCG CTG     1152
Gly Gly Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    370                 375                 380

CCG CTG CGC CTG GAC GCC ATC ACC GGC CCC GAG GAG GAA GGC GGG CGC     1200
Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
385                 390                 395                 400

CTG GAG ACC ATT CTC GGC TGG CCG CTG GCC GAG CGC ACC GTG GTG ATT     1248
Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                405                 410                 415

CCC TCG GCG ATC CCC ACC GAC CCG CGC AAC GTC GGC GGC GAC CTC GAC     1296
Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            420                 425                 430

CCG TCC AGC ATC CCC GAC AAG GAA CAG GCG ATC AGC GCC CTG CCG GAC     1344
Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        435                 440                 445

TAC GCC AGC CAG CCC GGC AAA CCG CCG CGC GAG GAC CTG AAG             1386
Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys
    450                 455                 460

TAA
```

Figure 24B

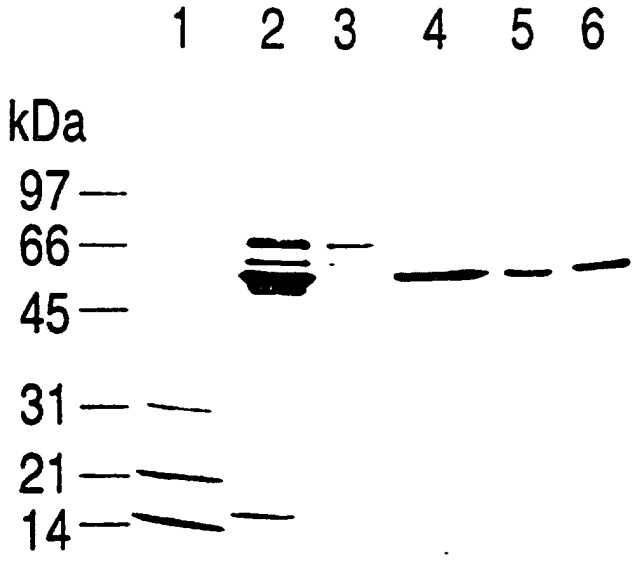


Figure 25

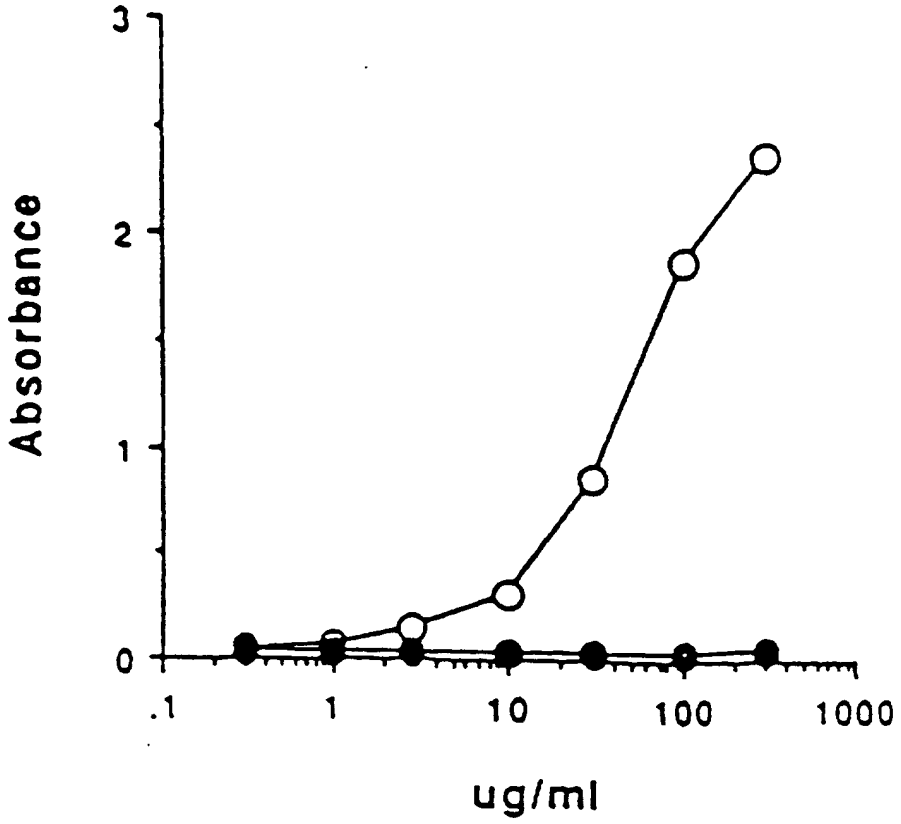


Figure 26

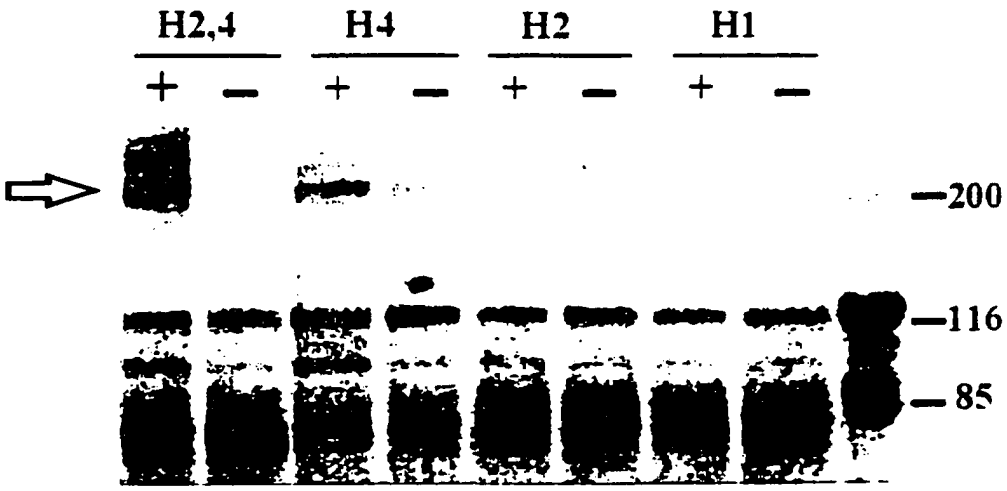


Figure 27

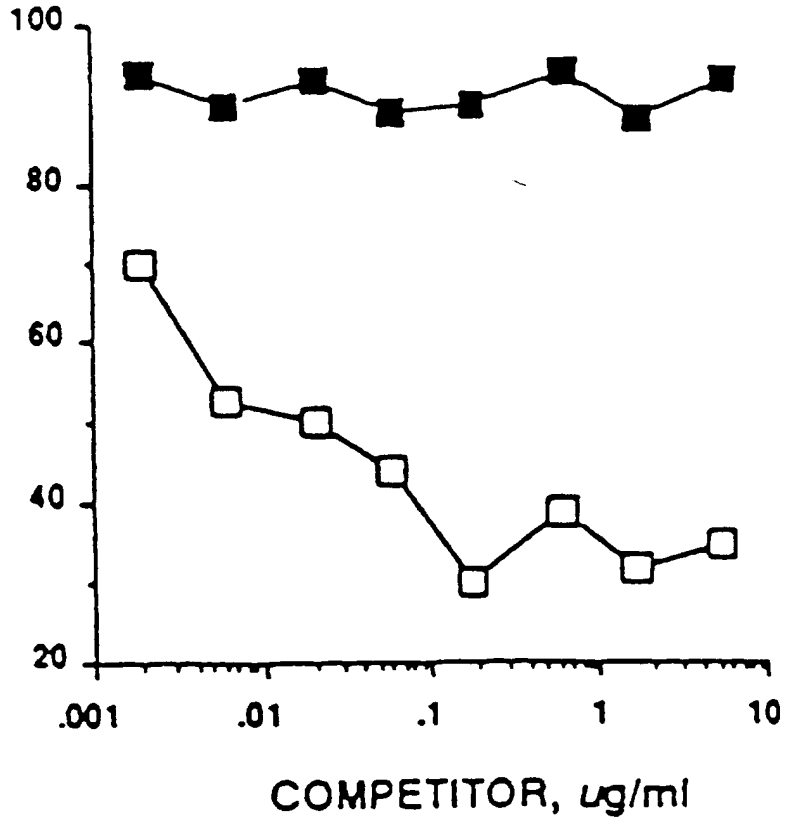


Figure 28A

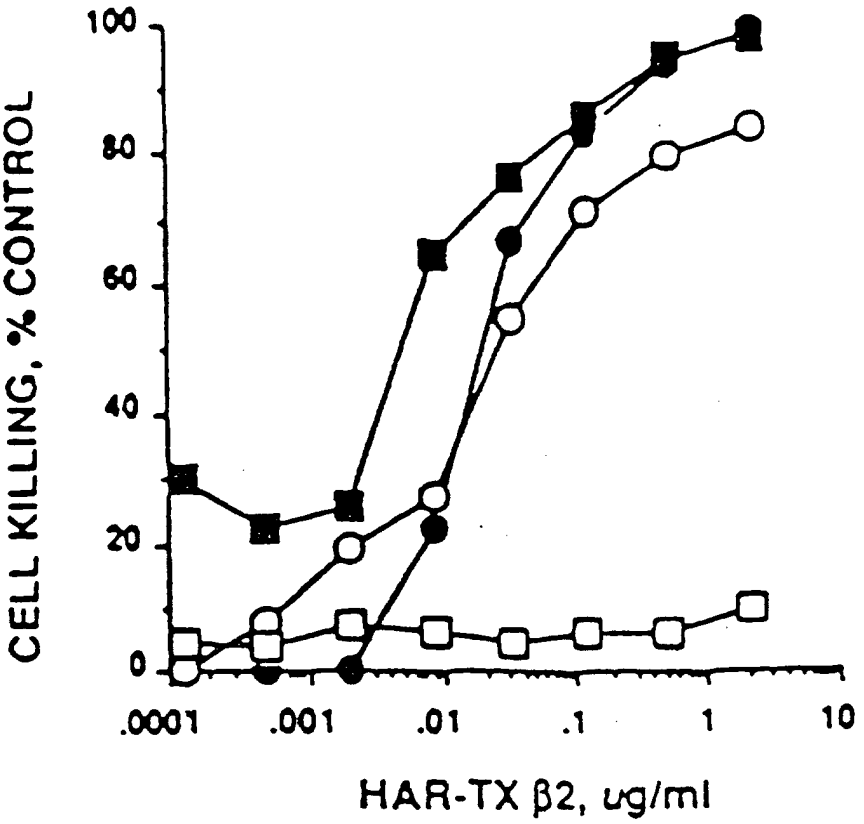

CELL KILLING, % CONTROL
100
80
60
40
20
0
.0001
.001
.01
.1
1
10
HAR-TX β2, ug/ml

Figure 28B

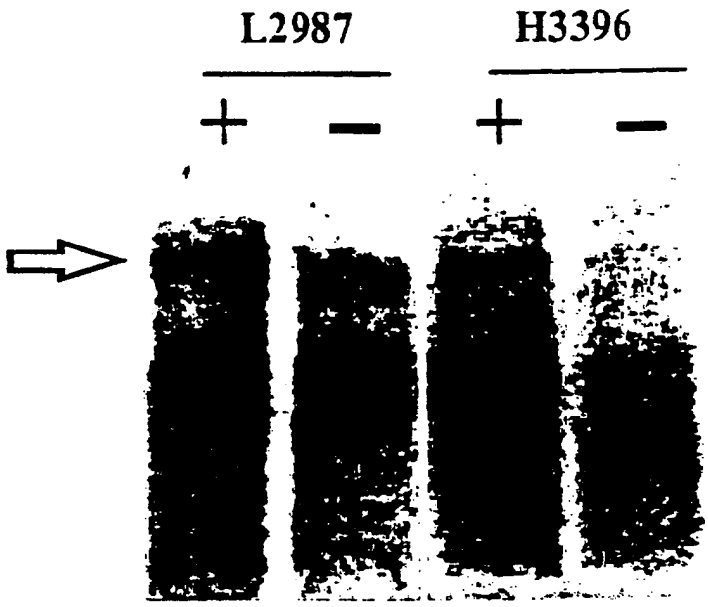


Figure 29